Dairy Cows

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Dairy Cows

Reproduction, Nutritional Management and Diseases

Catherine T. Hernandez
Editor
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In this book, the authors discuss the reproduction, nutritional management and diseases relating to dairy cows. Topics include strategies to improve the reproductive efficiency of dairy cattle; an illustrated classification system to define the causes of international bovine perinatal mortality; resetting the priorities for sustainable dairy farming under global change; and somatic cell count as a factor conditioning productivity of various breeds of cows and technological suitability of milk.

Chapter 1 - Currently there is no published classification system for the causes of death in cases of bovine perinatal mortality internationally. In addition, the criteria used to define these causes of death are also not standardised, nor published. This results in inconsistent reporting of many different causes of death and often a high proportion of cases being unexplained.

A system is required for codifying bovine perinatal mortality for epidemiological surveillance and perinatal audit, as in human perinatology where the World Health Organisation’s International Classification of Diseases Manual is used internationally. Hence, the objective of this study was to develop a novel classification system for both the criteria and the causes of death in cases of bovine perinatal mortality internationally in order to improve our understanding of the main causes of such reproductive loss. A foeto-maternal, clinico-pathological classification system was developed over a period of three years using three primary sources of information. A systematic literature-based review, the findings of an international Delphi survey and epidemiological and pathological data from an active surveillance, whole-herd necropsy study were used to design the system. Ten major causal categories of death were assigned with sub-classification as required; alphabetically –
combination of contributory factors (more than one cause of death), congenital
defect (economically lethal and lethal), dystocia (bradytocia, traumotocia,
bradytocia and traumotocia, dystocia anamnesis, dystoxia and fetal
maldisposition), eutoxia, haemorrhage and anaemia (external omphalorrhagia,
internal omphalorrhagia, idiopathic hemoperitoneum, anaemia), infection,
iiodine imbalance, premature placental expulsion, prematurity and other
specific disorders (e.g. accidental death, hypothermia, intra-uterine growth
retardation, etc.). Each cause of death was assigned a degree of confidence of
diagnosis from certain through probable to possible.

These causes of death were assigned using the written anamnesis from the
farmer or veterinary practitioner, gross necropsy observations (including
photo-documentation) and the associated laboratory tests, i.e. an algorithmic,
supply diagnosis. The cause of death indicated the pathological condition of
the fetus or calf which made the greatest contribution towards the death. Only
proximate (immediate) factors were listed in the cause of death, e.g. a genetic
mutation may be the ultimate cause of a lethal congenital defect but the defect
was the proximate cause of death. Some causes of death, e.g. dystocia, were
disaggregated in order to differentiate separate components (where a
portmanteau is used, e.g. dystoxia) which can be re-aggregated as necessary
for different reporting formats.

Differential diagnoses were reached through both processes of inclusion
and of exclusion. This ten-level classification system can be used to calculate
cause-specific mortality rates (CSMR) and the attributable fraction (AF) of
perinatal mortality due to each cause of death.

Chapter 2 – Global change increasingly affected agricultural production
and global community has begun to refocus in a change in livestock
production, defending the use of sustainable strategies. Considerations with
respect to changing environments should also address the dairy farm systems
and new goals have to be designed. Good farming practices should regard their
need for on-going adaptation to an ever-changing environment that should
offer solutions for buffering against climatic extremes, disease epidemics,
changing nutrient availability, seasonal availability of forage and other stresses
that will add to an already heterogeneous environmental condition. Sustainable
dairy systems should be adjusted to these new expectations, and be indeed
adapted to the new agricultural policies and the increasing demands of the
consumers for products free of drug residues (safety) and be more ecofriendly
produced. The key to success is to maximise farm efficiency finding the right
balance between the production system and the management techniques to
maximise the output for food production, involving a suitable dairy cow
biotype, which may trigger new strategies for feeding, breeding and health control whilst minimising impact on the environment and ensuring animal welfare and profitability for their business. This chapter book pretends to present a holistic capture of main issues regarding management, feeding regimes, breeding, reproductive efficiency with examples of current sustainable production systems.

Chapter 3 – Early detection of mastitis with subclinical symptoms is possible by determining somatic cell count (SCC). SCC is the most widely accepted indicator of the mammary gland health as well as milk quality and its technological suitability. The authors’ research has revealed that an increase of SCC (independently of a breed of cows) mainly causes a rise in a total crude protein content and a distinct reduction in lactose level (P≤0.01). Moreover, SCC also lengthens the time of milk enzymatic coagulation (P≤0.01) but it does not influence its thermal stability. Distinct negative relationship between casein content and SCC is confirmed by relatively high value of correlation coefficient (r=-0.59). In the authors’ studies the significant interactions (breed of cows x SCC) for the daily yield of cows, content of protein, casein and lactose, protein to fat ratio and rennet-induced milk coagulation time also have been stated, which indicates a differentiated response of various breeds of cows to udder inflammations. Holstein-Friesian cows are more sensitive to decline of daily yield, that is reflected in higher negative value of correlation coefficient between SCC and milk yield (-0.245). In Simmental and Jersey cows the correlations were negative as well but their values were substantially lower (r=-0.123 and r=-0.148) and statistically insignificant. With the age of cows increase in SCC was noted and in the cows of local breeds (Polish Red, Polish Black and White, Whitebacked) and Jersey that rise was much smaller in comparison to Polish Holstein-Friesian cows. Significant interaction (P≤0.05) for SCC between breed of cows and subsequent lactation was indicated. However, the significant changes in milk constituents were recorded only when the SCC exceeded 500 thous. ml\(^{-1}\), that is in milk that does not meet the current regulatory quality standards.

Somatic cell count also affects the changes in whey protein content. Rise of SCC decreased the content of major albumins, i.e. alpha-LA and beta-LG, by small degree, and that was confirmed by very low statistically insignificant correlation coefficients (r=-0.07 i r=-0.05). Negative value of both correlations, though, indicates a direction of changes and may imply that in more advanced stages of udder diseases the decrease of milk proteins is likely to be higher. However, with rise of SCC, content of immunoactive proteins (lactoferrin and lysozyme) as well as bovine albumin serum (BSA)
significantly increased. The significant impact of SCC on content of these proteins in milk is confirmed by relatively high positive values of computed correlation coefficients (lactoferrin $r=0.65$, lysozyme $r=0.63$ and BSA $r=0.59$). In the case of BSA that correlations were clearly differentiated in particular breeds of cows, i.e. $r=0.711$ for Holstein-Friesian, $r=0.577$ for Simmental and $r=0.472$ for Jersey. Thus, it can be assumed that there is a differentiated degree of permeability of mammary gland cell membranes in cows of various breeds.

Chapter 4 - The reproductive performance of a lactating herd is a major component of the profitability of a dairy farm. Factors such as negative energy balance, heat stress and failures in heat detection can severely compromise reproductive parameters. To overcome these problems, a variety of strategies can be used. For example, failures in estrus detection can be solved with the use of fixed-time artificial insemination (FTAI). Progesterone implants combined with estradiol administration are very effective in promoting the onset of a new follicular wave. With the use of a luteolytic agent and an inducer of ovulation, AI can be performed at the appropriate time without the need for estrus observation. In countries where the use of such drugs is not allowed, protocols based on GnRH and PGF2α may also offer optimal synchronization of ovulation. In locations where pregnancy rates are compromised by high temperatures, a viable alternative to FTAI may be the fixed-time embryo transfer (FTET). Embryos at the morula and blastocyst stage are more resistant to heat stress than gametes and embryos in early stages of development. Thus, embryo transfer (ET) on day 7 of development can ensure satisfactory pregnancy rates throughout the year, even in months and/or regions with higher average temperatures. ET has also been effective in preventing early embryonic mortality and increasing pregnancy rates in repeat breeders. Another strategy that can enhance the reproductive efficiency of dairy herds is the cryopreservation of embryos; embryos are stored and can be used at strategic times, such as in the warmer months of the year. This chapter will discuss technological strategies that can lead to higher breeding efficiency, improved reproductive efficiency and increased profitability of livestock on dairy farms.
Chapter 1

A NOVEL, ILLUSTRATED CLASSIFICATION SYSTEM TO DEFINE THE CAUSES OF BOVINE PERINATAL MORTALITY INTERNATIONALLY

John F. Mee*

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ABSTRACT

Currently there is no published classification system for the causes of death in cases of bovine perinatal mortality internationally. In addition, the criteria used to define these causes of death are also not standardised, nor published. This results in inconsistent reporting of many different causes of death and often a high proportion of cases being unexplained.

A system is required for codifying bovine perinatal mortality for epidemiological surveillance and perinatal audit, as in human perinatology where the World Health Organisation’s International Classification of Diseases Manual is used internationally. Hence, the objective of this study was to develop a novel classification system for both the criteria and the causes of death in cases of bovine perinatal mortality internationally in order to improve our understanding of the main causes of such reproductive loss. A foeto-maternal, clinico-pathological classification system was developed over a period of three years using three primary sources of information. A systematic literature-

* E-mail: john.mee@teagasc.ie.
based review, the findings of an international Delphi survey and epidemiological and pathological data from an active surveillance, whole-herd necropsy study were used to design the system. Ten major causal categories of death were assigned with sub-classification as required; alphabetically – combination of contributory factors (more than one cause of death), congenital defect (economically lethal and lethal), dystocia (bradytocia, traumotocia, bradytocia and traumotocia, dystocia anamnesis, dystoxia and fetal maldisposition), eutoxia, haemorrhage and anaemia (external omphalorrhagia, internal omphalorrhagia, idiopathic hemoperitoneum, anaemia), infection, iodine imbalance, premature placental expulsion, prematurity and other specific disorders (e.g. accidental death, hypothermia, intra-uterine growth retardation, etc.). Each cause of death was assigned a degree of confidence of diagnosis from certain through probable to possible.

These causes of death were assigned using the written anamnesis from the farmer or veterinary practitioner, gross necropsy observations (including photo-documentation) and the associated laboratory tests, i.e. an algorithmic, summary diagnosis. The cause of death indicated the pathological condition of the fetus or calf which made the greatest contribution towards the death. Only proximate (immediate) factors were listed in the cause of death, e.g. a genetic mutation may be the ultimate cause of a lethal congenital defect but the defect was the proximate cause of death. Some causes of death, e.g. dystocia, were disaggregated in order to differentiate separate components (where a portmanteau is used, e.g. dystoxia) which can be re-aggregated as necessary for different reporting formats.

Differential diagnoses were reached through both processes of inclusion and of exclusion. This ten-level classification system can be used to calculate cause-specific mortality rates (CSMR) and the attributable fraction (AF) of perinatal mortality due to each cause of death.

**INTRODUCTION**

Classification may be described as a construct systematically arranging similar entities with criteria or differing characteristics. Currently there is no classification system for bovine perinatal mortality. This contrasts with human perinatology where more than 35 classification systems have been published since the first system was developed in 1954 (Lawn et al., 2011).

Such systems attempt to establish the cause of inevitable/unavoidable death in an individual perinate, not the correlates of death in a population of perinates, such as, for example, maternal parity (Mee et al., 2008).
Classification attempts to define whether a perinate died of or due to a disorder (causal) or merely with a disorder (associative). The gradual evolution of these systems over time has resulted in the percentage of unexplained human stillbirths drop from 66% in the Wigglesworth system to only 15% in the ReCause of death (relevant condition at death) system (Gardosi et al., 2005). This demonstrates the potential for improved diagnosis rate in veterinary perinatology were an evolved classification system to be adopted. An improved diagnosis rate would in turn assist in encouraging higher carcass submission and hence necropsy rates, a major problem currently in the diagnosis of bovine perinatal mortality (Mee, 2008).

An ideal classification system for research and for routine diagnosis by veterinary pathologists for veterinary practitioners would identify the pathophysiologic entity initiating the chain of events that irreversibly led to death based on clinical, pathologic and laboratory data.

The criteria to be used to categorize a particular condition as a cause of perinatal mortality should consider the following principles: 1) there are epidemiologic data demonstrating a significant excess of perinatal mortality associated with the condition, 2) there is biologic plausibility that the condition causes perinatal mortality, 3) the condition is either rarely seen in association with live births or, when seen in live births, results in a significant increase in perinatal death, 4) a dose–response relationship exists so that the greater the “dose” of the disorder, the greater the likelihood of perinatal mortality, 5) the condition is associated with evidence of fetal compromise, and 6) the perinatal mortality likely would not have occurred if that condition had not been present, i.e., lethality.

When attempting to classify causes of perinatal mortality it is often difficult to determine the ‘definite’ cause of death. Risk factors may be significantly associated with perinatal mortality but can also occur in liveborn calves, multiple disorders can occur in the same calf and many cases cannot be explained. All perinatal classification systems suffer from these uncertainties (Reddy et al., 2009). One way of dealing with this inherent issue is to define each condition as a certain, probable or possible cause of death. In addition, the use of photographic images can assist in clarifying lesion assessment or scoring (Mee and Szenci, 2012).

The paucity of information on the classification of bovine perinatal mortality stems from the lack of any centralised coding of such deaths as exists for human perinatology, e.g. International Classification of Diseases (WHO, 2005). While national bovine laboratory data recording systems such as VIDA and FarmFile in the UK (Gibbens et al., 2008) and veterinary
diagnostic support systems such as the Cornell Consultant in the USA (www.vet.cornell.edu/consultant) exist they are not specialised perinatology classification systems.

The absence of an internationally accepted bovine classification system is a substantial barrier to any reliable audit of perinatal mortality causation and data meta-analysis including analysis of secular and geographical trends. Having a unified perinatal mortality causation mapping system would allow researchers, veterinary diagnosticians and veterinary practitioners share information in the same ‘language’ across laboratory, institutional and national boundaries. This would lead to a better understanding of perinatal mortality causation by farmers and veterinary practitioners, derive learning points for best clinical practice for students and provide a firmer foundation for prioritising interventions aimed at all-cause rate reduction by agri-stakeholders.

**Definition of Perinatal Mortality**

Bovine perinatal mortality may be defined as the death of a fullterm (≥260d gestation) fetus before, during or within 48h after calving (Mee, 2008a). In this definition fetal death is death prior to complete expulsion in the perinatal period. Stillbirth is birth after fetal death. Differentiation between the continuum of abortion and stillbirth is based on arbitrary gestational thresholds (e.g. <260 or 270 days) which are associated with the potential independent viability of the fetus (lower limits of viability). These bovine thresholds have been in use for over 150 years (Spencer, 1840). The data in Table 1 show the variability internationally in the definition of the term bovine ‘stillbirth’. An early attempt to standardise the nomenclature for bovine reproductive terms defined stillbirth as a full-term dead fetus and perinatal mortality as death from 42 days of gestation up to 28 days after birth (Anon, 1972). In human perinatology stillbirth is defined as death of a fetus weighing at least 500g or following a gestation of at least 22 weeks, showing no signs of life.

The data in Table 2 show the variability internationally in the definition of the term bovine ‘perinatal mortality’. In human medicine the term perinatal mortality includes stillbirths and early neonatal deaths up to 7 days of age. While these are the definitions recommended by the WHO (WHO, 2005), other national definitions are also used.
<table>
<thead>
<tr>
<th>Gestation threshold (days)</th>
<th>Postnatal threshold (hours)</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;260</td>
<td>12</td>
<td>USA</td>
<td>Linden et al., (2009)</td>
</tr>
<tr>
<td>&gt;270</td>
<td>24</td>
<td>UK</td>
<td>MAFF (1985)</td>
</tr>
<tr>
<td>265-295</td>
<td>48</td>
<td>USA</td>
<td>Meyer et al., (2001)</td>
</tr>
<tr>
<td>NR</td>
<td>48</td>
<td>Iran</td>
<td>Atashi (2011)</td>
</tr>
<tr>
<td>NR</td>
<td>24</td>
<td>France</td>
<td>Chassagne et al., (1999)</td>
</tr>
<tr>
<td>NR</td>
<td>24</td>
<td>Canada</td>
<td>Luo et al., (1999)</td>
</tr>
<tr>
<td>&gt;255</td>
<td>48</td>
<td>USA</td>
<td>Olson et al., (2009)</td>
</tr>
<tr>
<td>NR</td>
<td>24</td>
<td>Poland</td>
<td>Stefaniak et al., (2011)</td>
</tr>
<tr>
<td>&gt;260</td>
<td>0</td>
<td>Ireland</td>
<td>Mee, (2008a)</td>
</tr>
<tr>
<td>NR</td>
<td>48</td>
<td>USA</td>
<td>Cole et al., (2007)</td>
</tr>
<tr>
<td>&gt;260</td>
<td>24</td>
<td>Norway</td>
<td>Gulliksen et al., (2009)</td>
</tr>
<tr>
<td>&gt;240</td>
<td>1</td>
<td>Canada</td>
<td>Waldner et al., (2010)</td>
</tr>
</tbody>
</table>

NR - not reported.

<table>
<thead>
<tr>
<th>Gestation threshold (days)</th>
<th>Postnatal threshold (days)</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR</td>
<td>&lt;7</td>
<td>Mali</td>
<td>Wymann et al., (2006)</td>
</tr>
<tr>
<td>&gt;272</td>
<td>&lt;1</td>
<td>England</td>
<td>Brickell et al., (2009)</td>
</tr>
<tr>
<td>&gt;260</td>
<td>&lt;2</td>
<td>Ireland</td>
<td>Mee (2008a)</td>
</tr>
<tr>
<td>NR</td>
<td>&lt;1</td>
<td>Finland</td>
<td>Syrjala et al., (2007)</td>
</tr>
<tr>
<td>NR</td>
<td>&lt;1</td>
<td>Germany</td>
<td>Gundelach et al., (2009)</td>
</tr>
<tr>
<td>&gt;260</td>
<td>&lt;2</td>
<td>Canada</td>
<td>Khodakaram and Ikede (2005)</td>
</tr>
<tr>
<td>&gt;275</td>
<td>&lt;14</td>
<td>Japan</td>
<td>Ogata et al., (1999)</td>
</tr>
<tr>
<td>&gt;260</td>
<td>&lt;2 hours</td>
<td>Ireland</td>
<td>Collery et al., (1996)</td>
</tr>
<tr>
<td>&gt;260</td>
<td>&lt;2</td>
<td>USA</td>
<td>Johanson et al., (2011)</td>
</tr>
<tr>
<td>260-330</td>
<td>&lt;1</td>
<td>Switzerland</td>
<td>Bleul (2011)</td>
</tr>
<tr>
<td>&gt;260</td>
<td>&lt;1</td>
<td>Germany</td>
<td>Hoedemaker et al., (2010)</td>
</tr>
</tbody>
</table>

NR - not reported.
Causes of Bovine Perinatal Mortality

There appears to be some agreement in the literature on the common causes of bovine perinatal mortality (Table 3). The major causes of bovine perinatal mortality, as described in recent necropsy studies internationally, are anoxia (approximately 50%) and dystocic trauma (approximately 25%) and, to a much lesser extent, other causes (approximately 15%), infections (approximately 10%) and congenital defects (approximately 5%), (Table 3). On average, some 20% of cases have no diagnosed cause but this varies between 0 and 70% between studies (Table 3). The variation in the proportions of necropsy-diagnosed causes of death reflects variations in the causative risk factors but also variations in diagnostic definitions and the number and selection criteria for calves and herds examined.

While the cause of death is usually determined from a necropsy examination it is recognised that in some cases pathognomonic lesions may not be visible at necropsy, e.g. accidental non-parturient trauma or premature placental expulsion. Hence, a clinico-pathological diagnosis is often used to assign weight to the clinical signs or anamnesis as well as the necropsy findings. This may explain why some cases of bovine perinatal mortality are classified as unexplained where there is an inadequate history supplied with the carcass.

Within studies on bovine perinatal mortality the number of calves or herds investigated as well as any exclusion criteria, (e.g. heifers’ calves only, beef calves only, singletons only), also affect the proportion of bovine perinatal mortality caused by any particular cause of death. In addition to these differences in diagnostic criteria and study design, the attributable fraction of bovine perinatal mortality caused by any particular cause of death may be predetermined by the surveillance system implemented. In passive surveillance only extreme cases may be voluntarily submitted to the laboratory unsolicited due to cost or inconvenience resulting in a non-random sample of the population at risk. With risk-based, targeted or whole-herd active surveillance a more representative sample of the population at risk may be collected.

Thus there is wide variation in the number of causes of death diagnosed in different calf studies internationally (Table 4) suggesting ad hoc recording of the cause of death. It must be recognised that these studies differed in diagnostic criteria and in design, both of which can contribute to differences in the number of causes of death recorded. By comparison, 11 cause of death
categories, each with sub-classes, are listed by the WHO for perinatal mortality in babies; maternal factors, disorders of fetal growth, birth trauma, respiratory and cardiovascular disorders, infections, haemorrhagic and haematological disorders, endocrine and metabolic disorders, digestive system disorders, temperature regulation disorders, other disorders and congenital malformations, (WHO, 2005). These categories are widely used internationally often with some modifications. For example, in Ireland these categories can be collapsed into 5 mega-categories (maternal factors, immaturity, respiratory/cardiovascular disorders, congenital malformations and all other specific causes) (ESRI, 2012). In addition, a standardised protocol has been developed recently on how to conduct an autopsy in cases of human stillbirth (Pinar et al., 2011).

These observations indicate the need for standardisation of assignable causes of death in cases of bovine perinatal mortality as previously proposed (Mee, 2009).

Table 3. Necropsy-diagnosed causes of death (%) for calves dying in the perinatal period internationally (1990-2010)

<table>
<thead>
<tr>
<th>Country</th>
<th>Calves (No.)</th>
<th>Dystocia</th>
<th>Anoxia</th>
<th>Congenital defects</th>
<th>Infection</th>
<th>Other</th>
<th>Unknown</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Belgium</td>
<td>100</td>
<td>7</td>
<td>55</td>
<td>6</td>
<td>18</td>
<td>4</td>
<td>10</td>
<td>De Kruijf and Benedictus (1993)</td>
</tr>
<tr>
<td>Canada</td>
<td>560</td>
<td>40.2</td>
<td>NR</td>
<td>4.3</td>
<td>2.9</td>
<td>31</td>
<td>21.6</td>
<td>Waldner et al., (2010)</td>
</tr>
<tr>
<td>Denmark</td>
<td>130</td>
<td>9</td>
<td>81</td>
<td>1.5</td>
<td>8</td>
<td>0.1</td>
<td>0</td>
<td>Agerholm et al., (1993)</td>
</tr>
<tr>
<td>Finland</td>
<td>148</td>
<td>43</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>29</td>
<td></td>
<td>Syrjala et al., (2007)</td>
</tr>
<tr>
<td>Iceland</td>
<td>129</td>
<td>34</td>
<td>37</td>
<td>NR</td>
<td>12</td>
<td>13</td>
<td>3.9</td>
<td>Siguroarson et al., (2007)</td>
</tr>
<tr>
<td>Ireland</td>
<td>119</td>
<td>34</td>
<td>1</td>
<td>14</td>
<td>9</td>
<td></td>
<td>42</td>
<td>Collery et al., (1996)</td>
</tr>
<tr>
<td>Japan</td>
<td>155</td>
<td>21</td>
<td>NR</td>
<td>3.9</td>
<td>NR</td>
<td>5.1</td>
<td>69.7</td>
<td>Ogata et al., (1999)</td>
</tr>
<tr>
<td>Netherlands</td>
<td>193</td>
<td>36.8</td>
<td>8.3</td>
<td>7.3</td>
<td>5.6</td>
<td>42</td>
<td></td>
<td>Muskens and Vos (2008)</td>
</tr>
<tr>
<td>Nr Ireland</td>
<td>365</td>
<td>23</td>
<td>46</td>
<td>NR</td>
<td>31</td>
<td>NR</td>
<td></td>
<td>McCoy et al., (1997a)</td>
</tr>
<tr>
<td>Sweden</td>
<td>76</td>
<td>46.1</td>
<td>NR</td>
<td>5.3</td>
<td>2.6</td>
<td>10.5</td>
<td>35.5</td>
<td>Berglund et al., (2003)</td>
</tr>
<tr>
<td>USA</td>
<td>60</td>
<td>25</td>
<td>28.5</td>
<td>3.3</td>
<td>5</td>
<td>6.6</td>
<td>31.6</td>
<td>Schefers (2009)</td>
</tr>
</tbody>
</table>

Dairy and beef calves, all others are dairy calves unless superscripted ¹; ² NR=not recorded, ³ Beef calves; ⁴ Anoxic and dystocic lesions combined, ⁵ these calves had shorter gestation and lower birth weights.
Table 4. Number of cause of death categories (excluding undetermined) in necropsy studies of bovine perinatal mortality internationally

<table>
<thead>
<tr>
<th>Causes of death (No.)</th>
<th>Calves (No.)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>560</td>
<td>Waldner et al., (2010)</td>
</tr>
<tr>
<td>10</td>
<td>43</td>
<td>Essmeyer (2006)</td>
</tr>
<tr>
<td>7</td>
<td>8,995</td>
<td>Kirkbride, (1992)</td>
</tr>
<tr>
<td>7</td>
<td>492</td>
<td>Bellows et al., (1987)</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>De Kruif and Benedictus (1993)</td>
</tr>
<tr>
<td>6</td>
<td>119</td>
<td>Collery et al., (1996)</td>
</tr>
<tr>
<td>5</td>
<td>130</td>
<td>Agerholm et al., (1993)</td>
</tr>
<tr>
<td>5</td>
<td>129</td>
<td>Siguroarson et al., (2008)</td>
</tr>
<tr>
<td>5</td>
<td>293</td>
<td>Smyth et al., (1992)</td>
</tr>
<tr>
<td>5</td>
<td>148</td>
<td>Syrjala et al., (2007)</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>Schefers (2009)</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>Khodakaram-Tafti and Ikede, (2005)</td>
</tr>
<tr>
<td>4</td>
<td>193</td>
<td>Muskens and Vos (2008)</td>
</tr>
<tr>
<td>4</td>
<td>76</td>
<td>Berglund et al., (2003)</td>
</tr>
<tr>
<td>3</td>
<td>155</td>
<td>Ogata et al., (1999)</td>
</tr>
</tbody>
</table>

Criteria Used to Define Causes of Perinatal Mortality

The foregoing indicates that there is limited consensus on the common causes of bovine perinatal mortality. In addition, the criteria used to assign causes of death vary considerably between published studies. For example, while Khodakaram-Tafti and Ikede, (2005) used the history of dystocia and detection of traumatic lesions, Collery et al., (1996) used the history of dystocia or posterior presentation with or without the detection of traumatic lesions to assign this cause of death. In other studies only the detection of traumatic lesions was used (Agerholm et al., 1993, Smyth et al., 1992). Thus while dystocia may be a commonly diagnosed cause of death, as the criteria used to define ‘dystocia’ vary between studies, logically the proportion of bovine perinatal mortality attributed to dystocia (the attributable fraction) will vary also.

Similarly, while meconium staining of the hair coat and subserosal haemorrhages has been induced experimentally by anoxia in the bovine foetus (Dufty and Sloss, 1977), in some studies only meconium staining was used as a criterion to diagnose anoxia (Collery et al., 1996) while in other studies
subserosal haemorrhages and tracheal congestion and mucus have been used, but not meconium staining (Wilsmore, 1989).

For other causes of death, e.g. intrauterine growth retardation (IUGR) there is no consensus on which criteria are diagnostic. Some authors have used body weight (Murray, 1990, Orgeig, 2010) or organ weights (Steinhardt et al., 1993), others crown rump length (Collery et al., 1996, Sharpe, 1998) and others compact bone percentage (Dwyer, 1991), presence of radiodense growth retardation lines in long bones (Smyth and Ellis, 1996) or bone lengths (Richardson 1978).

These observations indicate that there are no minimal standards for bovine perinatal necropsy and the need for standardisation of criteria used to assign causes of bovine perinatal mortality. Hence, the objective of this study was to develop a novel classification system for both the criteria and the causes of death in cases of bovine perinatal mortality internationally in order to improve our investigations of the understanding of the main causes of such reproductive loss.

**MATERIALS AND METHODS**

A foeto-maternal, clinico-pathological classification system to define the criteria for, and the causes of, bovine perinatal mortality was developed over a period of three years (2010-2012) using three primary sources of information. A systematic literature review (Mee, 2013), the findings of an international Delphi survey (Mee et al., in press) and epidemiological and pathological data from a whole-herd necropsy study (Mee, 2013) were sequentially used to design the system.

**1. Literature Review**

Initially all publications where a necropsy-derived diagnosis of bovine perinatal mortality was recorded, with or without diagnostic criteria, in the last 25 years (1987-2012) were retrieved from the author’s personal files and electronic databases by cross-referencing sourced publications.

The information in these publications was extracted and tabulated, e.g. Tables 3 and 4. In addition, publications from other species (e.g. humans, primates, sheep, pigs, horses, dogs) with relevant information were included, particularly where experimental models of causes of death produced
pathological lesions. A total of 173 publications are cited here from this literature review. The findings from this literature review are reported in detail by Mee (2013).

2. Delphi Survey

In order to elicit current consensus from veterinarians about both the criteria and the causes of death in cases of bovine perinatal mortality internationally two groups of veterinarians [subject matter experts (SME) and non-SMEs] were contacted in a Delphi survey in 2012. The SMEs were selected on the basis of their scientific publications or experience of working in a veterinary diagnostic or research laboratory in the area of bovine perinatal mortality.

The non-SMEs were self-selected as cattle veterinarians without particular expertise in bovine perinatology. A total of 74 veterinarians (46 SMEs and 28 non-SMEs) from 23 countries responded. The study was conducted using Delphi methodology over seven rounds. Respondents were asked to agree the causes of bovine perinatal mortality and for each cause to agree the supporting diagnostic criteria. The findings from this study are reported in detail by Mee et al., (in press).

3. Necropsy Study

A prospective, longitudinal, active surveillance necropsy study was carried out in 30 Irish dairy herds over three years (2010-2012) by the author. Herd- and animal-level epidemiological data were collected and complete necropsy examinations, including gross pathology, histopathology, bacteriology, serology and virology, were carried out on 680 carcasses from foetuses and calves which died before, during or within 48 hours after calving following a gestation period of at least 260 days. For cases of indeterminate gestational age (e.g. unrecorded natural services) parity and fetal plurality-adjusted birth weight was used as a surrogate criterion using the third percentile (mean−2SD) as the lower inclusion limit (depending on the dam and sire breeds and whether the calf was a singleton or a twin); the same principle used by the WHO to define human perinatal mortality cases (Lawn et al., 2011).
Data from an independent dataset where gestation length and birth weight were recorded were used to establish the variation in birth weight of full-term calves (Table 5). For Jersey or Jersey × cows mated to Jersey or Jersey × bulls an inclusion threshold of ≥15 kg was used for singletons or twins.

For Jersey or Jersey × dams or sires mated to non-Jersey or Jersey × dams or sires, respectively, (i.e. only the dam or the sire was Jersey or Jersey ×, not both) an inclusion threshold of ≥20 kg was used for singletons or twins. For other dairy breed dams (non-Jersey or Jersey ×) mated to other dairy sires (non-Jersey or Jersey ×) or beef sires, inclusion thresholds of ≥25 and ≥20 kg were used for singletons and multifetal pregnancies, respectively.

All necropsy information was recorded by the same prosector (the author) on a dictaphone and photo-documentation of lesions was carried out as the case indicated. The findings from this study are reported in detail by Mee (2013).

When the results of the literature review and the Delphi survey responses were applied to the necropsy database the causes of death were ranked in order of individual cause incidence, i.e. where more than one cause of death was recorded for a calf (combination cause of death category) both were included in the individual cause of death incidence database.

Table 5. Birth weights (kg) (mean, mean+2SD, range), gestation length (days) and number of records for fullterm (260-300 days), singleton (n=10,422) and twin (n=615) calves from nine Teagasc research dairy herds over a twenty year period (1991-2011)

<table>
<thead>
<tr>
<th>Dam genotype</th>
<th>Sire genotype</th>
<th>Singletons</th>
<th>Twins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean</td>
<td>Mean+2SD</td>
</tr>
<tr>
<td>Jersey, JerseyX or dairy</td>
<td>Jersey, JerseyX or dairy</td>
<td>32.63</td>
<td>20.31-44.95</td>
</tr>
<tr>
<td>Dairy (excl. Je, JeX) *</td>
<td>Dairy (excl. Je, JeX)</td>
<td>41.84</td>
<td>29.61-54.07</td>
</tr>
<tr>
<td>Dairy (excl. Je, JeX)</td>
<td>Beef</td>
<td>42.82</td>
<td>30.86-54.78</td>
</tr>
</tbody>
</table>

* Dairy (excl. Je, Jex) = Holstein-Friesian, Friesian, Friesian x, Ayrshire, Norwegian Red, Norwegian Red x, Swedish Red, Swedish Red x, Montbeliarde, Montbeliarde x, Normande, Normande x; Beef = all recorded beef breeds.
The causes of death with an incidence ≥5% were assigned individual categories and the other causes of death were aggregated into an ‘other specific disorder’ category. Following completion of these iterations the classification system was refined for each cause of death and its subcategories, with each criterion contributing to a certain, probable or possible cause of death. Certain referred to a condition which unequivocally was the cause of death, probable referred to a condition which with high likelihood caused death and possible to a condition which with reasonable certainty was involved in a pathophysiologic sequence that led to death.

RESULTS

Causes of Perinatal Mortality in 680 Dairy Calves

When the causes of death were originally classified following the Necropsy Study (Mee, 2013) the three most common causes of death were: a combination of causes of death, dystocia and eutoxia. When the combination cause of death category was disaggregated the three most common causes of death were dystocia, lethal congenital defects and eutoxia (Table 6).

Definitions of Cause of Death

The cause of death was assigned using the Literature Review, Delphi survey and the Necropsy Study (written history from the farmer, the gross necropsy observations and the laboratory tests), i.e. an algorithmic, summary diagnosis.

This is a foeto-maternal clinicopathological classification system. The main cause of death indicates the pathological condition of the fetus or calf which made the greatest contribution towards the death (ESRI, 2012). Only proximate (immediate) factors are listed in the cause of death, e.g. a genetic mutation may be the ultimate cause of a lethal congenital defect but the defect was the proximate cause of death. Some cause of death, e.g. dystocia, are disaggregated in order to differentiate separate components (where a novel portmanteau is used, e.g. dystoxia) which can be re-aggregated as necessary for different reporting formats. The causes of death are assignable, presumptive, mutually exclusive causes of death based on the evidence available on the factors which contributed to death in each case. Differential
diagnoses of cause of death are reached through both processes of inclusion, e.g. detection of a significant lesion, and of exclusion, e.g. absence of diagnostic criteria – unexplained perinatal mortality. The causes of death can be used to calculate cause-specific mortality rates (CSMR) and the attributable fraction (AF) of PCM due to each cause of death.

**Table 6. Prevalence of individual * causes of perinatal mortality**
**(in alphabetical order) in 680 dairy calves over three years (2010-2012)**

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>% of calves</th>
<th>Rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>Congenital defect</td>
<td>20.3</td>
<td>2</td>
</tr>
<tr>
<td>Dystocia</td>
<td>51.6</td>
<td>1</td>
</tr>
<tr>
<td>Eutoxia</td>
<td>14.0</td>
<td>3</td>
</tr>
<tr>
<td>Haemorrhage or Anaemia</td>
<td>8.1</td>
<td>7</td>
</tr>
<tr>
<td>Infection</td>
<td>12.2</td>
<td>4</td>
</tr>
<tr>
<td>Iodine imbalance</td>
<td>6.5</td>
<td>9</td>
</tr>
<tr>
<td>Other specific disorder</td>
<td>7.5</td>
<td>8</td>
</tr>
<tr>
<td>Premature placental expulsion</td>
<td>12.1</td>
<td>5</td>
</tr>
<tr>
<td>Prematurity</td>
<td>9.4</td>
<td>6</td>
</tr>
<tr>
<td>Unexplained</td>
<td>12.2</td>
<td>-</td>
</tr>
</tbody>
</table>

* combination of causes of death category is disaggregated so % figures do not add up to 100%.

Following sequential aggregation of these three primary sources of information (literature review followed by Delphi survey followed by necropsy results) ten major causal categories of death were assigned with sub-classification as required; alphabetically – combination of contributory factors (more than one cause of death), congenital defect (economically lethal and lethal), dystocia (bradytocia, traumotocia, bradytocia and traumotocia, dystocia anamnisis, dystoxia and fetal maldisposition) eutoxia, haemorrhage and anaemia (external omphalorrhagia, internal omphalorrhagia, idiopathic hemoperitoneum, anaemia), infection, iodine imbalance, premature placental expulsion, prematurity and other specific disorders (e.g. accidental death, hypothermia, intra-uterine growth retardation, etc.). These 10 causes of death are defined hereunder in alphabetical order along with their sub-classifications and brief commentaries.

1. **Combination of Contributory Factors**

Multiple causes of death are diagnosed in the same calf. Hence, if a lethal congenital defect was diagnosed along with an infectious cause of death both
were combined into the combination cause of death category as it is not always possible to assign which is the actual cause of death. This category includes certain, probable and possible causes of death depending on the combination involved.

2. Congenital Defect (Lethal and Economically-Lethal)

Grossly visible structural defects including lethal congenital defects recorded by body system such as hydrocephalus, hydranencephaly, and economically-lethal congenital defects (necessitating euthanasia) such as anal atresia, intestinal atresia (Figure 1), (Mee and Kennelly, 2010), enterocoele, omphalocoele (Mee, 1994), vestigial limbs, severe arthrogryposis (Leipold et al., 1993), anasarca, severe cardiac defects, central nervous system defects (Cornillie et al., 2004), palatoschisis, chondrodysplasia (Mee, 1995), or combinations of these lethal defects, but not exclusively this list. Where diagnostic facilities exist, this category also includes non-visible/occult lethal congenital defects such as metabolic storage disorders, e.g. citrullinaemia. This category includes certain and probable causes of death depending on the defect.

3. Dystocia

The term dystocia is used to mean a difficult or abnormal calving (Hafez, 1980) encompassing traumotocia, bradytocia, traumotocia and bradytocia combined, dystocia anamnesis, dystoxia and maldisposition in calves which died during or after birth; each of these sub-classifications is defined below.

Traumotocia: severe antemortem lesions (haemorrhage at the lesion site) consistent with iatrogenic (resulting from the action of the farmer or veterinary practitioner) parturient trauma. For example, fractured spine/vertebral luxation (Figure 2) (Agerholm et al., 1993), fractured ribs (costal and costo-chondral junctions) (Schuijt, 1990), fractured mandible (Trent and Ferguson, 1985), limb fractures (Ferguson et al, 1990), moderate and severe subcutaneous thoracic and limb haemorrhages/bruising, haemoarthrosis, hepatic or renal rupture, moderate and severe haemoperitoneum (Waldner et al., 2010), diaphragmatic hernia (excluding hiatus hernia), (SAC, 2012a) or polytrauma.

These lesions were detected in calves born at assisted calvings (iatrogenic trauma) with (traumotoxia – trauma and anoxia combined in the same calf) or without anoxic lesions or subcutaneous bruising at site of calving ropes placement on legs, and without bradytocia. This is a certain or probable cause of death.
Figure 1. Atresi jejuni (type 1) showing marked distension of the proximal jejunum with meconium (economically-lethal congenital defect).

Figure 2. Fractured spine at the thoraco-lumbar junction with extensive haemorrhage (traumotocia).
Figure 3. Marked capital and lingual swelling due to subcutaneous oedema (bradytocia).

**Bradytocia**: moderate or severe subcutaneous antemortem oedema (e.g. legs, tongue, capital, cervical; Figure 3); prolonged stage two of calving; Everett-Hincks et al., 2007, Khodakaram-Tafti and Ikede, 2005, Gee et al., 1989), (excluding serosanguinous subcutaneous oedema following death and retention in utero) with or without a written anamnesis of prolonged stage one of calving (e.g. uterine inertia, milk fever/clinical hypocalcaemia, ‘slow calving syndrome’, disturbance during calving, incomplete cervical dilatation, uterine torsion; Collery et al., 1996), calving assistance score ≥3 (0-5 scale) or anoxic lesions (bradytoxia) and without traumotocia. This is a probable cause of death.

**Traumotocia and bradytocia**: combination of traumatic and bradytocic lesions with calving assistance score ≥2 (0-5 scale), with or without anoxic lesions. This is a certain or probable cause of death.

**Dystocia anamnesis**: history of a difficult calving [assistance score ≥3 (0-5 scale)], (Collery et al., 1996), (without maldisposition, traumotocia, bradytocia or dystoxia) or other abnormal calving history, e.g. uterine torsion, uterine rupture. This is a probable or possible cause of death depending on the anamnesis.

**Dystoxia (dystocia with anoxia)**: calving assistance score ≥3 (0-5 scale), with at least two locations of moderate or severe anoxic lesions. For example, meconium stained hair coat or in lungs (Collery et al., 1996, Schefers, 2009, Alonso-Spilsbury et al., 2005), ecchymotic (3-10 mm) or suffusive subserosal
haemorrhages on internal organs, e.g. tracheal mucosa, heart (epicardium, endocardium), pleura, thymus, adrenal glands, sclera (Gavin and Zackery, 2011, Hey et al., 1986) or meningeal congestion (Dufty and Sloss, 1977, Haughey, 1991). These lesions were detected in the presence or absence of gingival cyanosis (Collery et al., 1996) and without traumotocia or bradytocia lesions (Salihagic-Kadic, et al., 2006). This is a certain or probable cause of death depending on the extent of the calving difficulty and lesions.

**Maldisposition:** Fetal mis-alignment (malpresentation, malposition or malposture, entwined/co-presented twins) with a calving assistance score of $\geq 2$ (0-5 scale), in the absence of traumotocia, bradytocia or anoxia (Collery et al., 1996). This is a certain, probable or possible cause of death depending on the maldisposition and degree of calving difficulty.

**4. Eutoxia (Eutocia with Anoxia)**

Calving assistance score $\leq 2$ (0-5 scale), with at least two locations of moderate or severe anoxic lesions [meconium stained hair coat or in lungs, ecchymotic suberosal haemorrages on internal organs, e.g. tracheal mucosa (Figure 4), heart (epicardium, endocardium), pleura, thymus, sclera, adrenal glands or meningeal congestion (Figure 5)] in the presence or absence of gingival cyanosis and in the absence of dystocia (e.g. traumotocia, bradytocia, maldisposition). Synonyms for this category of mortality include anoxia of unknown origin, non-visible dystocia (e.g. non-visible delayed stage one of calving) and idiopathic anoxia. This is a probable or possible cause of death depending on the extent of lesions.

**5. Haemorrhagic and Haematological Disorders**

This category refers to haemorrhage and anaemia in or from the foetus or perinate with the following sub-classifications.

**Internal omphalorrhagia:** moderate or severe haemoperitoneum or severe peri-umbilical arterial haematoma (Figure 6) with at least one unsealed umbilical artery in the absence of trauma or other source of haemorrhage (Vanholder et al., 2001, Rademacher, 2006). This is a certain cause of death.

**External omphalorrhagia:** moderate or severe blood staining of the hair coat from the umbilicus with anaemia and at least one unsealed umbilical artery in the absence of hemoperitoneum, severe peri-umbilical arterial haematoma and trauma with or without blood-staining of the calving site (Meydan et al., 2009, Kamimura et al., 2001, Bentinck-Smith et al., 1960). This is a probable or possible cause of death depending on the extent of haemorrhage.
Figure 4. Extensive haemorrhage in the tracheal submucosa (anoxia).

Figure 5. Severe meningeal congestion (anoxia).
Figure 6. Severe bilateral peri-umbilical artery haematomata (internal omphalorrhagia).

Figure 7. Marked pallor of the entire carcass (anaemia).
Hepatic rupture associated-haemoperitoneum: moderate or severe haemoperitoneum with subcapsular hepatic rupture in the absence of omphalorrhagia and trauma (idiopathic hepatic rupture, e.g. hypovitaminosis E; Green et al., 1995). This is a certain cause of death.

Idiopathic haemoperitoneum: moderate or severe haemoperitoneum in the absence of omphalorrhagia, hepatic rupture or trauma (e.g. rupture of the aorta or gastrointestinal vessel mesentery complex or severed vessels in enterocoele or kidney; DAFM, 2011a, possible bacterial infection: VLA, 2010 or melaena, SAC, 2012b). This is a probable or possible cause of death depending on the extent of haemorrhage.

Anaemia only: severe anaemia [pallor of conjunctiva and at least three other organs, e.g. skeletal muscles, thymus, tracheal mucosa, brain, liver, heart, lungs or kidneys, (Figure 7)] with or without gingival pallor in the absence of haemoperitoneum, omphalorrhagia, hepatic rupture or other source of haemorrhage or trauma (e.g. BVDv infection; Bell, 2011, twin-to-twin transfusion; WHO, 2005, neonatal isoerythrolysis; Dowssett et al., 1978, anaemia of unknown origin; SAC, 2011a, Ogata et al., 1999). This is a probable or possible cause of death depending on the extent of anaemia.

6. Infection

Examples of prenatal (congenital) and postnatal infections are detailed hereunder though other foetopathogens may also be listed depending on their relevance nationally, e.g. Coxiella burnetii, Mycoplasma/Ureaplasma, Parachlamydia, Bluetongue virus, Schmallenberg virus, etc...

Bovine herpes virus-1: demonstration of viral antigen (e.g. isolation, PCR; DAFM, 2011b), presumptive histopathological lesions (e.g. nonsuppurative focal hepatic necrosis; Kirkbride, 1992) or antibodies (e.g. ELISA positive titre, excluding weak positive/inconclusive titres) in fetal blood where the calf had not consumed colostrum. This is a probable or possible cause of death depending on the detection of lesions/antigen or antibodies, respectively.

Bovine viral diarrhoea virus: antigen detected in fetal tissues (e.g. ear, spleen, lungs, thymus) or blood (e.g. by PCR, AG-ELISA or virus isolation; Graham et al., 2009, Muskens and Vos, 2008) preferably with gross or histopathological lesions. This is a certain, probable or possible cause of death depending on the supporting evidence from lesions.

Leptospira serovar Hardjo: detection of the bacterium (e.g. PCR or immunofluorescence; Smyth et al., 1999, Collery et al., 1996) or antibodies detected in fetal blood (e.g. ELISA, MAT, Immunocomb) where the calf had
not consumed colostrum. This is a probable or possible cause of death depending on the detection of antigen or antibodies, respectively.

*Neospora caninum*: demonstration of antigen (e.g. IHC), detection of presumptive histopathological lesions (e.g. encephalitis, myocarditis) or detection of antibodies (positive titre, excluding weak positive/inconclusive titres) in fetal blood (e.g. ELISA, indirect fluorescent antibody test; Graham et al., 1996) where the calf had not consumed colostrum, with or without neurological clinical signs. This is a probable or possible cause of death depending on the detection of lesions/antigen or antibodies, respectively.

*Primary foetopathogen*: Examples include *Bacillus licheniformis*, *Trueperella pyogenes*, *Listeria monocytogenes*, *Histophilus somni*, *Mannheimia haemolytica*, *Salmonella Dublin* cultured (or detected by PCR) in pure or nearly pure growth from the abomasal contents/fetal tissues/placenta (AFBI/DAFM, 2011, AHVLA, 2011a, SAC, 2012c, VLA, 2011), or diagnosed by microscopy (fungi, e.g. *Aspergillus* spp.; Kirkbride, 1992) or by demonstration of toxin (e.g. *Clostridium perfringens* ELISA; Watson and Scholes, 2009). For other possible pathogens, e.g. *E coli*, in addition to culture-positive results, lesions (gross or histopathological) should be present (DAFM, 2012a).

Figure 8. Fibrinous pericarditis (infection).
Because the placenta is frequently contaminated, isolation from the placenta should be supported by gross or histopathological lesions. This is a certain or probable cause of death depending on the supporting evidence from lesions.

**Gross or histological lesions**: indicative of infection, e.g. pericarditis (Figure 8), multi-focal necrotising meningoencephalitis, haemorrhagic emphysematous abomasitis, omphalo-peritonitis, chorio-amnionitis, omphalo-sepsis, peritonitis, pleuropneumonia, pneumonia, systemic sepsis, pyothorax, pneumonoenteritis or enteritis, (Waldner et al., 2010, DAFM, 2012b, 2011c, AHVLA, 2011b). There may or may not be low serum immunoglobulin status (e.g. Zinc Sulphate Turbidity test value <15 units). This is a certain, probable or possible cause of death depending on the extent of the lesions.

**7. Iodine Imbalance**

Histopathologically abnormal thyroid morphology [moderate or severe microfollicular hyperplasia (Figure 9) or macrofollicular hypoplasia; (‘colloid goitre’) is the ‘gold standard’ for diagnosis of iodine imbalance in a perinate (La Perle, 2012). Other findings consistent with iodine imbalance include absolute goitre (thyroid gland weight ≥30g; Mee, 1993, Smyth et al., 1996), (Figure 10) or relative goitre (thyroid gland weight/body weight ratio ≥0.70; Knowles and Grace, 2007) usually found with abnormal thyroid morphology, significant thyroid biochemistry (low iodine content) and associated pulmonary pathology, e.g. atelectasis (Smyth et al., 1996). This is a probable or possible cause of death depending on the detection of histological/biochemical or thyroid weight abnormalities, respectively.

*Figure 9. Severe microfollicular thyroid hyperplasia (iodine imbalance).*
Figure 10. Absolute goitre (58.3g thyroid with comparator 6.9g thyroid), (iodine imbalance).

8. Premature Placental Expulsion (PPE)

Calf born with entire placenta attached (Figure 11) based on written anamnesis (placenta emerged before or with the fetus) or necropsy observation, with or without visible anoxic lesions (as some foetuses are dead in utero for some time prior to calving), (Mee, 1991a, Sluijter et al., 1990). This is a certain cause of death.

Figure 11. Calf with entire placenta attached (premature placental expulsion).
9. Prematurity

Where gestation length is known, calf is born before physiological maturity (singletons ≤270 days and twins ≤265 days gestation) (Roberts, 1986). Where gestation length is unknown, birth weight is below the breed and plurality-adjusted range for term calves (Table 5).

![Figure 12. Small (24.7kg) 267 day gestation fetus with light hair coat (prematurity).](image)

Where gestation length and birth weight is unknown, at least two of these findings: small calf, short light hair coat (Figure 12), partially erupted incisor teeth (Figure 13), domed skull (in the absence of brain abnormalities) with or without anamnestic information [respiratory distress syndrome (RDS), muscle weakness, floppy ears, or delayed reflexes in the calf and/or lack of maternal udder swelling or pelvic ligament relaxation precalving and/or sudden unexpected calving preterm].

Though the underlying cause of the premature birth may or may not be diagnosable, this is a probable or possible cause of death depending on the degree of prematurity.
10. Other Specific Disorders

It is not possible to be exhaustive in including all possible causes of death in separate categories so a heterogeneous category was created to record other uncommonly assigned causes of death (<5% individual cause of death incidence). Examples include placentitis (Waldner et al., 2010), hyperthermia/heat stress (Yates et al., 2012), neoplasia (Kirkbride, 1992), hyposelenosis/myocardial myopathy (Waldner et al., 2010, Murray et al., 2008, Siguroarson et al., 2008), intoxications, for example nitrate toxicity (‘blue baby syndrome’), (Ozmen et al., 2005, Johnson et al., 1994), uterine haemorrhage, hormonal imbalances (Sorge et al., 2008), maternal endotoxaemia (Kirkbride, 1992) and maternal euthanasia/death (Waldner et al., 2010), accidents (DAFM, 2011d), hypothermia (Olson et al., 1980, 1981), IUGR (Ogata et al., 1999) and iatrogenic causes. This is a certain, probable or possible cause of death depending on the specific disorder.

In the necropsy database analysed here the following three causes of death occurred individually in approximately 4% (accident), 1% (hypothermia) and 3% (IUGR) of cases.

Accidents

Two sub-classifications are included in this category.

Non-parturient trauma: death caused by being stood on or attacked by cow (assault injury) or injured in the calving environment, e.g. by automatic passage scraper in cubicle/freestall house. Diagnosis based on written
anamnesis and traumatic lesions, e.g. fractured ribs and haemothorax (Figure 14), (DAFM, 2011d), exsanguinations arising from hepatic rupture (DAFM, 2012c), usually in the absence of subcutaneous bruising (Munro and Munro, 2011) and in the absence of iatrogenic traumotocia. This is a certain, probable or possible cause of death depending on the history and lesions detected.

Colostrum aspiration: death due to administration of colostrum by oro-oesophageal tube. Diagnosis based on written anamnesis (history of failure to suck, ‘stomach-tubing’, dyspnoea, weakness, depression, and recumbence) and presence of colostrum in the trachea and bronchial tree, pulmonary oedema and congestion and focal pulmonary consolidation. Note some calves die before pneumonia develops. If they survive long enough a diffuse suppurative/necrotising bronchopneumonia, particularly of the apical lobes, develops with foreign basophilic material and bacterial colonies in the airways (DAFM, 2012d). This is a certain or probable cause of death depending on the history and lesions detected.

Hypothermia

History of extreme cold weather (ambient temperature close to 0°C, high wind speed, rain/sleet/snow, i.e. wind chill factors), unobserved calving, and calf not born into a straw bed (e.g. born in the cubicle/freestall house passageway pushed out by automatic scrapers, un-bedded tie-stall or outdoors) and not dried off (wet or faeces-covered coat; abandoned by dam), combined usually with distal limb subcutaneous haemorrhages and oedema, particularly of the hind limbs (Olson et al., 1980, 1981, Haughey, 1973) and no voluntary colostrum intake (abomasum containing mucus only). This is a probable or possible cause of death depending on the history and lesions detected.

Intrauterine Growth Retardation (IUGR)

Abnormally low breed and plurality-adjusted body weight in the third percentile (<mean-2SD kg), (Table 5) in a full-term (>95% of gestation length, i.e. not premature) calf (Richardson, 1978), (Figure 15). For example, a body weight of 20kg or less and a gestation period of 275 days or more was used by Ogata et al., (1999) to diagnose IUGR in Japanese Black stillborn calves. Synonyms for IUGR include small for gestational age (SGA) and dysmature. Possible causes include acute (e.g. summer mastitis; Richardson and Terlicki, 1980) or chronic maternal systemic infection or debilitating disease with or without placentitis (e.g. Bacillus licheniformis, paratuberculosis; Scott, 2011, Bluetongue virus; Richardson et al., 1985) and maternal undernutrition (Wu et
al., 2009). As these underlying causes are often difficult to differentially diagnose in the fetus, IUGR is a possible cause of death.

Figure 14. Severe haemothorax after the dam kneeled on her calf (accidental non-parturient trauma).

Figure 15. Abnormally small (12.2kg) for date full-term (289day) fetus (intrauterine growth retardation).
**Table 7. Causes of death, listed alphabetically, categorised by potential lethality (certain, probable and possible)**

<table>
<thead>
<tr>
<th>Cause of death</th>
<th>Levels</th>
<th>Certain</th>
<th>Probable</th>
<th>Possible</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combination</td>
<td>Multiple</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Congenital defect</td>
<td>Multiple</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Dystocia</td>
<td>Traumotocia</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bradytocia</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Traumotocia and bradytocia</td>
<td>✓</td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Dystocia anamnesis</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dystoxia</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maldisposition</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Eutoxia</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Haemorrhage and anaemia</td>
<td>Internal omphalorrhagia</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>External omphalorrhagia</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hepatic-rupture-haemoperitoneum</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Idiopathic-haemoperitoneum</td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anaemia</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Infection</td>
<td>Antigen detected</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Lesions diagnosed</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Antigen and lesions</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Antibodies detected</td>
<td></td>
<td>✓</td>
<td></td>
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<tr>
<td></td>
<td>Antibodies and lesions</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Iodine imbalance</td>
<td>Histopathology</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Goitre (absolute/relative)</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Histopathology and goitre</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Low thyroid iodine</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
<tr>
<td>Premature placental expulsion</td>
<td></td>
<td>✓</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prematurity</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Other disorders</td>
<td>Non-parturient trauma</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>Colostrum aspiration</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hypothermia</td>
<td></td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IUGR</td>
<td></td>
<td></td>
<td>✓</td>
</tr>
</tbody>
</table>

**Certain, Probable and Possible Causes of Death**

The classification of causes of death into certain, probable and possible causes of death is illustrated in Table 7. Given the complexity of bovine perinatal mortality, it is not possible to be prescriptive in each and every case of death so these severity bands are merely categorical guidelines indicating the potential lethality of each cause of death. In the final analysis, the ten
causes of death may be grouped into certain (PPE), certain or probable (congenital defect), probable or possible (eutoxia, iodine imbalance, prematurity) and certain, probable or possible (combination cause of death, dystocia, haemorrhage or anaemia, infection, other specific disorder) depending on the circumstances and findings in each individual case.

**Unexplained Perinatal Mortality**

If none of the criteria above are met following thorough epidemiological, clinical and necropsy investigations the cause of death is undetermined. This is a diagnosis of exclusion in the absence of assignable cause of mortality; ‘idiopathic perinatal mortality or unexplained stillbirth’. Examples of findings include death in utero of unknown origin (autolysed foetus), an anamnesis of eutocia only with normal fetal alignment, multifetal pregnancy only, nonlethal congenital defects, minor pathogens isolated from abomasal contents (e.g. *Streptococcus* spp., *Staphylococcus* spp., *Bacillus* spp., *Gardanellera* spp. or mixed bacterial growth), sparse anamnesis (e.g. calf found dead) or grossly no visible lesions (NVL) at necropsy. In some cases it may not be possible with routine diagnostics in veterinary laboratories to detect the cause of death, e.g. karyotypic abnormalities and inborn errors of metabolism. In other cases the absence of the placenta may hinder diagnosis, e.g. placentitis.

**DISCUSSION**

The classification system developed here seeks to establish what went wrong, not necessarily why. It does not necessarily identify the underlying risk factors associated with the cause of death (Mee et al., 2008). It is novel in accepting more than one cause of death in the same calf as multiple causes of death are common in bovine perinatal mortality. While it attempts to establish the cause of death in an individual perinate these findings, unlike in human perinatology, need to be interpreted also at the herd-level in order to effect change in loss rates.

Central to the classification system is a thorough necropsy examination. While the level of agreement between non-necropsy and necropsy-based diagnosis can be high for some other conditions, e.g. bloat and respiratory disease (Anspaugh et al., 2010), this is not necessarily the case in perinatal mortality. For example, in one study verbal autopsy under-diagnosed IUGR
and prematurity in human perinates (Marsh et al., 2003) and, in another, autopsy led to a change in diagnosis in 13% and additional findings in 26% of human perinatal deaths (Cartlidge et al., 1995). Infection and congenital defects were most commonly under-diagnosed in the latter study. In an earlier study autopsy was the only means of establishing a cause of death in 26% of human perinatal deaths when compared with clinical assessment (Meier et al., 1986). Similar studies have not been published for calves. However, adoption of routine all-carcass necropsy examinations has been proposed as an essential procedure to encourage preventive medicine amongst farming clients (Lester, 1987). In addition to the findings from a gross necropsy examination, for some disorders additional laboratory tests are essential for diagnosis, e.g. infections.

While a necropsy is essential to this classification system so also is the clinical history of the circumstances surrounding the calf’s death, usually the calving details. Collection of these details can be difficult in routine submissions to veterinary diagnostic laboratories as the person submitting the carcass may not know these details. While for some causes of death this is not important, e.g. isolation of a foetopathogen with gross lesions, for others, e.g. hypothermia, this information is critical. Thus this is a clinico-pathological classification system relying on both strands of information to assist in diagnosis.

The depth of diagnostic investigation employed for each case of perinatal mortality often depends on the reported incidence of the problem at a herd-level. Thus if only a single case is submitted and only a single case has occurred some diagnostic tests may not be applied without supporting evidence, e.g. testing of body fluids and organs for multiple infections. But where such evidence exists, further analyses are conducted. Where a herd problem is presented more extensive investigation will be carried out.

In drawing up this list of causes of death and the criteria used to assign same there were many alternative causes and criteria considered both from the literature review and in particular the Delphi survey. Hereunder some of the discussion around these topics is presented for each cause of death alphabetically.

**Congenital Defect**

Some congenital defects may not be lethal *per se* but are ‘economically-lethal’. For example, a calf born with a cleft palate or a vestigial limb or arthrogryposis can live but will not survive in an intensive dairy herd and will
be euthanized. These sub-lethal cases were included in this category. While some congenital defects are easy to define as lethal, e.g. holoacardius amorphous (Mee, 1990), others are less clear-cut, e.g. intestinal atresia, because some affected calves can survive following reconstructive surgery (Azizi et al., 2010). However, they are not independently viable. It is also accepted that while some calves may be diagnosed with a congenital defect and die in the perinatal period, e.g. ventricular septal defect, other calves with this defect may survive longer. These may be described as defects which significantly compromise viability and a normal productive life. As many congenital defects are occult, e.g. anencephaly, a necropsy examination is essential for this diagnosis. No attempt was made to differentiate hereditary from non-hereditary defects as the ultimate aetiology of many defects is unclear, particularly multiple defects.

**Dystocia**

This category is much broader than the traditional definition of dystocia often used in epidemiological studies, e.g. by Mee et al., (2011), as only a difficult assisted calving. Here it encompasses traumotocia, bradytocia, traumotocia and bradytocia combined, dystocia anamnesis, dystoxia and maldisposition. In this way two of the most common diagnoses in perinatal mortality, anoxia and dystocia can be combined (dystoxia) whereas anoxia associated with eutocia is categorised separately (eutoxia). Similarly, different types of dystocia, e.g. bradytocia and traumotocia, which commonly occur together do not need to be differentiated but can be coded as a separate subclass. Calves presented with a history of difficult or prolonged calving but without traumatic lesions are included here also as these are not uncommon findings. This can occur where a mechanical calving aid has not been used or where calving has been unduly prolonged and the fetus dies in utero. In such cases dystocia (abnormal calving) is present but traumatic lesions may not be present in the calf. Many such cases have supporting clinical information such as an oversized calf, vaginal lacerations or a downer cow. It was not possible to be prescriptive about the duration of a prolonged calving as the frequency of observations is so variable and the observable signs of the onset of calving are also highly variable between animals.
Eutoxia

Eutoxia may be caused by utero-placental circulatory disturbances (e.g. prolonged period of uterine contractions), placental insufficiency (e.g. partial separation, placentitis, and dysfunction), umbilical cord disorders (e.g. torsion, compression, entanglement, stretching or premature rupture) or maternal conditions (e.g. anaemia, incomplete cervical dilatation). Pathological lesions associated with anoxia have been recorded in experimental animal models (e.g. Raju, 1992, Haughey, 1980, Dufty and Sloss, 1977, Glastonbury, 1977) and in numerous observational studies (e.g. Muskens and Vos, 2008, Alonso-Spilsbury et al., 2005, Gill, 2001, Collery et al., 1996, Smyth et al., 1992). These lesions include meconium staining, ecchymotic subserosal haemorrhages, visceral or meningeal congestion and gingival cyanosis. These lesions do not in themselves cause death but are patho-markers for hypoxic-ischaemia/respiratory and metabolic acidosis and terminal encephalopathy (Buckmann and Pattinson, 2009). In human perinatology ‘asphyxia’ is diagnosed clinically from the Apgar score, cord pH and at autopsy from the lesions outlined above (Becher et al., 2004, Wigglesworth, 1998). Autopsy lesions include cerebral congestion, congested viscera, visceral haemorrhages and meconium aspiration (Jezowa and Feit, 2011, Pinar, 2004, Wigglesworth, 1998). In the case of meconium aspiration syndrome (meconium, squames and keratin debris in alveoli possibly with mild interstitial pneumonia; Schoon and Kikovic, 1987) and severe skin meconium staining, this may reflect the onset of the fetal distress syndrome prior to the onset of stage two of parturition (Motas-Rojas et al., 2006, Mee, 1991b, Miller, 1988). Although these lesions occur in cases of perinatal mortality, individually they may have a low diagnostic sensitivity, e.g. 20% (specificity 85%) for meconium staining (any degree of) in caesarean-delivered calves (Schuijt, 1992), whether acidotic or not (Szenci et al., 1989). This indicates that the presence of any degree of meconium staining alone cannot predict perinatal viability. Similarly, low sensitivities have been reported for individual anoxia lesions in human perinates (Becher et al., 2004).

Haemorrhage or Anaemia

Perinatal mortality due to haemorrhage or anaemia is uncommon, and without necropsy, can be difficult to diagnose clinically, and so is not listed in some textbooks (e.g. Jubb and Kennedy, 1963). However, numerous examples
of different types of bovine perinate haemorrhage and anaemia have been reported in the literature, e.g. DAFM, 2011a, Rademacher, 2006, Kamimura et al., 2001, Vanholder et al., 2001, Ogata et al., 1999. Haemorrhage and haematological disorders are also listed as a cause of death in other species, e.g. babies (ESRI, 2012) and lambs (Dwyer, 1991).

**Infection**

As it is not always possible to be sure when infection occurred, pre-, intra- and postnatal infections were combined in this category. Additionally, it is not possible to be definitive about whether the presence of infection caused death, e.g. BVDv-positive feti can survive to be PI calves, the presence of lesions can be obscured by autolysis or missed by sub-sampling for histopathology and the presence of lesions, antibodies or detection of a fetopathogen does not automatically mean causality. In some cases, e.g. infection with *Bacillus licheniformis*, the lesions/organism may be present in the placenta and not in the fetus and so a negative fetal culture result cannot rule out infection as a cause of death. For some infections, e.g. *Salmonella Dublin*, maternal serology can be useful to predict fetal infection from a single sample with a moderate degree of certainty (Sanchez, et al., 2011). All infections are not equally pathogenic and some may interact. There is no consensus on a definitive list of primary (cause disease as a result of their intrinsic virulence within the healthy pregnant animal) and secondary opportunistic (cause disease in pregnant cattle with depressed resistance) fetopathogenic infections. Hence, the need for more than one piece of evidence of infection when assigning causality. Despite these limitations, infection is a commonly listed cause of perinatal mortality in all species, e.g. calves (Muskens and Vos, 2008), babies (ESRI, 2012), lambs, (Sharpe, 1998) and foals (Smith et al., 2003).

**Iodine Imbalance**

If dietary iodine imbalance persists for long enough or is severe enough it will cause hypothyroidism which in turn will lead to thyroid malfunction. This may cause perinatal mortality via retarded fetal development (pulmonary, cerebral, and skeletal), reduced surfactant production or impaired thermogenesis (Grace and Knowles, 2010). For example, in calves hypothyroidism has been associated with goitre, pulmonary atelectasis and fetal
pneumonia (Smyth et al., 1996), hyaline membrane formation (Grunert et al., 1992) and increased susceptibility to hypothermia (Vermorel and Vernet, 1985). However, as most of these are non-specific disorders, iodine imbalance can only be diagnosed in dead calves where thyroid abnormalities are found (e.g. AHVLA, 2011c, 2012, SAC, 2011b, van Wuijckhuise et al., 2003). Abnormal thyroid histopathology is considered the gold standard when diagnosing iodine imbalance in perinates, however, congenital dysshormonogenetic goitre, though rare, displays similar lesions (La Perle, 2012). Histology allows differentiation between current and historical dietary iodine imbalance as goitre persists when iodine deficiency has been corrected (Livesey and Payne, 2007). There are numerous ancillary tests to diagnose iodine imbalance (thyroid, plasma, milk or urine iodine content) and hypothyroidism (thyroxine). Goitre and relative goitre have traditionally been used to diagnose thyroid malfunction in regions with a high incidence of bovine perinatal mortality (Jamieson et al., 1945), but, the diagnostic thresholds for both can vary (e.g. Suttle, 2004, Synge, 1982, Hernandez et al., 1972). In order to prioritise which glands to sample for histology or iodine content, goitre is often used as a criterion for further testing (e.g. VIDA, DAFM). However, goitrous thyroid glands do not always have histological lesions indicative of iodine imbalance (e.g. due to oedema, haemorrhage or autolysis) and non-goitrous glands are not always euplastic (Mee, 1993, Smyth et al., 1996). Given these limitations, abnormal thyroid weight (goitre or relative goitre) cannot be used alone to diagnose iodine imbalance as a cause of bovine perinatal mortality. In addition, experimental reproduction of simple iodine deficiency alone while producing abnormalities in thyroid histology and mass did not induce stillbirths in selenium-adequate heifers (McCoy et al., 1997b). But, it is known that the effects of simple iodine deficiency may be compensated by adequate selenium status (Zagrodzki et al., 1998, Mee, 1996). This suggests that while iodine deficiency alone causes hypothyroidism, other contributory factors, including possibly the duration and severity of iodine and selenium deficiency, may be needed to cause iodine deficiency disorders (Livesey and Payne, 2010). Given these caveats, in clinical veterinary practice abnormal thyroid histopathology in conjunction with the response to micronutrient supplementation is often used as diagnostic criteria (e.g. Wither, 1997, Mee et al., 1995, Seimiya et al., 1991, Rogers, 1992). Iodine imbalance is considered an uncommon cause of death in calves and in other species also, e.g. in lambs (Dwyer, 1991) and in babies (ESRI, 2012).
Premature Placental Expulsion (PPE)

In many cases the premature expulsion of the placenta may be because of a delay in expulsion of the fetus and so the placental detachment and expulsion may not be premature per se but it does precede or accompany fetal expulsion. This may be induced by factors which do not stop the onset of calving but do prevent the delivery of the fetus, e.g. uterine inertia or fetal malposition (Mee, 1991a).

However, cases may occur where parturition is not delayed but the placenta is prematurely separated (abruption) and expelled, e.g. placentitis or premature/induced calving (MacDiarmid, 1983). This condition is an example where an anamnesis is critical to diagnosis as although lesions of anoxia may be found in the fetus they are not always visible and may be obscured by autolysis where the fetus is retained in utero.

Prematurity

Given the continuum from immaturity through prematurity to maturity and postmaturity all viability thresholds are arbitrary whether in bovine (Roberts et al., 1986) or in human perinatology (WHO, 2005) or in other species.

However, while in humans single ‘genotype’ criteria are used, in cattle breed-specific gestational thresholds may be needed, though these do not currently exist across breeds nor would they account for individual fetal maturity-for-age trajectories.

In cases of indeterminate gestational age, surrogate indices have been proposed, e.g. birth weight or crown-rump length (CRL). The latter metric is susceptible to differing measurement techniques which can affect its external validity and its correlation at term with gestational age is only moderate (Richardson, et al., 1990).

In addition to these limitations, some premature calves can survive despite neurological (reduced suck reflex, in-coordination, dullness, dysphagia) and pulmonary immaturity (RDS) with good nursing care and therapy (Bleul, 2010) but have a higher neonatal mortality rate. Possible causes of prematurity include pharmaceutically induced parturition, pre-term elective Caesarean section, pre-natal calf cropping, twinning and infections causing pre-term calving.
Other Specific Disorders

While there is a multitude of possible causes of perinatal mortality, many of these conditions occur at a very low incidence and so they were included in this heterogeneous category. Some are reliant on a good anamnesis (e.g. accidents, hypothermia, hyperthermia, maternal toxaemia or euthanasia/death), recording of fetal body weight and gestation length (IUGR), require submission of the placenta (placentitis) or have indefinite diagnostic criteria (e.g. nitrate toxicity, heat stress, hyposelenosis and hormonal imbalances). While alternative diagnostic criteria can be used, e.g. in calves of indeterminate gestational age IUGR can be diagnosed from growth arrest lines in long bones by radiography; these may have a low yield in stillborn calves (Smyth and Ellis, 1996).

Classification System Critique

Comparison of the results from this system with those previously published (Table 3) reveals some interesting findings. Firstly, this system identified a combination of causes of death as the most common cause of death category. This is a novel finding and contrasts with the data in the literature where anoxia is generally the most common cause of death and the ‘other’ category ranks approximately third (Table 3). This difference occurs because in the present system where another cause of death in addition to anoxia is diagnosed, e.g. a lethal congenital defect, this is recorded as a combination cause of death. Another major difference is the increase in the diagnostic sensitivity in the current system. More causes of death are recorded here than in most recent published reports where this varies between 3 and 16, with an average of 6 cause of death categories (Table 4). This allows relatively uncommon causes of death, such as haemorrhage and premature placental expulsion to be more ‘visible’ amongst the data whilst not having too many categories which may render their use confusing or complex.

The higher ranking of lethal congenital defects in this system than in many previous studies (Table 3) probably reflects the study design (active whole-herd surveillance) and higher incidence of individual defects (e.g. intestinal atresia; Mee and Kenneally, 2010) in this dairy cattle population. Hence the relative ranking of the causes of death reported here may not be valid in other study designs (e.g. passive surveillance) or cattle populations. This is true for other reports also where local risk factors affect the causes of death, e.g. a high
incidence of skeletal degenerative myopathy in a Canadian study (Waldner et al., 2010), a high incidence of IUGR in a Japanese study (Ogata et al., 1999), a high incidence of micronutrient disorders in an Icelandic study (Hardarson, 2012) and a high incidence of premature calves in heat stress zones internationally (Yates et al., 2012).

In order to be of greatest use to the end users, i.e. veterinary pathologists, practitioners and farmers, ideally a classification system will have a minimal number of cases unexplained (Chan et al., 2004). In the current system less than 15% of cases were unclassifiable and so were categorised as unexplained. A comparison with recent studies (Table 3) shows that this figure varies widely from 0 to 70% with an average of approximately 20%. This indicates that the present system is slightly better than average, and markedly better in comparison to some studies, in assigning a cause of death. These figures are comparable with those in human perinatology (Gardosi et al., 2005). However, the real value and limitations of this novel system will not be realised until it is used by veterinarians.

There are many limitations in this novel system. Perhaps one of the biggest obstacles to its benefits is the current ambivalence of many farmers, veterinary practitioners and veterinary diagnosticians towards the value of perinatal necropsy. This results in low carcass submission rates for cases of fullterm bovine perinatal mortality (Mee, 2008b). In addition, the system does not include information on placental pathology. Although submission of the placenta with the dead fetus is best practice this rarely occurs with fullterm calves, as opposed to aborted feti, and so was not included as a routine diagnostic specimen. This is a clinico-pathological system which relies partially on anamnestic information from the farmer or veterinary practitioner which may be incomplete, unreliable or absent. It does not encompass advanced diagnostic methodologies for some conditions, e.g. congenital defects. While such techniques are available for some malformations in some research or diagnostic laboratories, they are not widely available internationally. But where they are they will add value to the diagnosis rate. Similarly, calf carcasses or organs are rarely weighed in veterinary diagnostic laboratories currently so information on reference body weights or organ: body weight ratios are not used. This system may encourage targeted weighing of carcasses or organs where this is integral to better diagnostics.

The ease of use of the system will vary between veterinary diagnosticians depending on the focus of their work, e.g. a research audit versus a busy diagnostic laboratory workflow.
Similarly, inter-observer variability will inevitably exist in criterion recording, hence the inclusion of exemplar photographic images here to improve this level of concordance. Taxonomic issues may arise when a classification system is transposed across language barriers. All of these limitations will result in some degree of inaccurate assignment of a classification.

This system will need to be revised, refined and updated based on the requirements of individual users, possible changes in population risk factors and the development of new diagnostic techniques. Thus it would be expected to evolve, similar to the human perinatology classification systems, over the years.

**CONCLUSION**

It is concluded from the literature review that currently there is no published classification system for the causes of death in cases of bovine perinatal mortality internationally. It is concluded from the international Delphi survey that the criteria used to define these causes of death are also not standardised. It is concluded from the necropsy study that the causes of death in cases of bovine perinatal mortality can be aggregated into ten major causal categories with sub-classification as required; combination of contributory factors (more than one cause of death), congenital defect (economically lethal and lethal), dystokia (bradystocia, traumotocia, bradytocia and traumotocia, dystokia anamnesis, dystoxia and fetal maldisposition) eutocia, haemorrhage and anaemia (external omphalorrhagia, internal omphalorrhagia, idiopathic hemoperitoneum, anaemia), infection, iodine imbalance, premature placental expulsion, prematurity and other specific disorders (e.g. accidental death, hypothermia, intra-uterine growth retardation, etc.). Each cause of death could be assigned a degree of confidence of diagnosis from certain through probable to possible. This ten-level classification system can be used to calculate cause-specific bovine perinatal mortality rates.

**ACKNOWLEDGMENTS**

The author thanks the veterinarians internationally who contributed generously of their time to respond to the Delphi survey. In particular, thanks to Andy Holliman, AHVLA, UK whose enquiries stimulated the preparation of this manuscript.
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Reviewed by: Andrew Holliman, BVMS, MRCVS.
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Chapter 2

RESETTING PRIORITIES FOR SUSTAINABLE DAIRY FARMING UNDER GLOBAL CHANGING

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ABSTRACT

Global change increasingly affected agricultural production and global community has begun to refocus in a change in livestock production, defending the use of sustainable strategies. Considerations with respect to changing environments should also address the dairy farm systems and new goals have to be designed. Good farming practices should regard their need for on-going adaptation to an ever-changing

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environment that should offer solutions for buffering against climatic extremes, disease epidemics, changing nutrient availability, seasonal availability of forage and other stresses that will add to an already heterogeneous environmental condition. Sustainable dairy systems should be adjusted to these new expectations, and be indeed adapted to the new agricultural policies and the increasing demands of the consumers for products free of drug residues (safety) and be more ecofriendly produced. The key to success is to maximise farm efficiency finding the right balance between the production system and the management techniques to maximise the output for food production, involving a suitable dairy cow biotype, which may trigger new strategies for feeding, breeding and health control whilst minimising impact on the environment and ensuring animal welfare and profitability for their business. This chapter book pretends to present a holistic capture of main issues regarding management, feeding regimes, breeding, reproductive efficiency with examples of current sustainable production systems.

1. SUSTAINABILITY IN THE AGROECOSYSTEM

Today, the dairy sector is among the most industrialised livestock sectors worldwide. However, marked differences are found between countries and production areas (IFCN Dairy Report, 2010). In the West, dairy farms are run in accordance with industrial production models. Competitiveness depends on increased output and enhanced economic efficiency. This has prompted an ongoing search for economies of scale, intensification of production, mechanisation, labour specialisation and rapid technological change (Wilson, 2001). Developments over the last 100 years have also served to highlight the real and potential damage to the environment caused by dairy farming (Styles and Jones, 2007; Sevenster and De Jong, 2008). There is a growing awareness that the resources on which progress is based are by no means infinite. Therefore, many difficult decisions have been taken regarding the most appropriate use of resources in the light of environmental degradation.

Long-term development requires dairy systems to be both economically competitive and environmentally sustainable. A reductionist view of the issue would lead to the adoption of “weak” sustainability, which seeks to ensure the viability of the system by maintaining the total capital stock from generation to generation. In this approach, economic growth is fully compatible with
environmental conservation, since natural capital and man-made capital can substitute each other through technological progress (Perman et al., 2003).

However, the relationships between the various elements of dairy systems are considerably more complex. If we agree that uncertainty regarding the functioning of environmental processes and the risk of irreversibility of ecosystems outweigh economic efficiency criteria, then we are accepting the “strong” sustainability approach (Constanza and Patten, 1995), highlighting the complementarity rather than the replaceability of man-made capital and natural capital.

The viability is thus based on a complex interaction of two dynamic systems: the socioeconomic system and the ecosystem. That interaction involves an ongoing process of coadaptation in which the resilience of the system as a whole plays a crucial role (Fiksel, 2006). The overall resilience of a system is the degree to which disturbances can be absorbed or assimilated into the dynamics of that system. In dairy systems, resilience is associated with the natural services offered by the ecosystem to the agrosystem, such as the circulation of nutrients and the supply of water (Grimm and Wissel, 1997). Any imbalance in these services with respect to the agrosystem requires external changes to be made in order to ensure that resilience of the system is maintained.

If a dairy system aim to persist over time, it must also be socially convenient, i.e. it must provide societal added values apart from the products themselves (Shreck et al., 2006). The achievement of social sustainability (Vallance et al., 2011) should pass through the three main aspects of “what people need”, “what is good for the environment”, and “what people want”. The concept of social sustainability was also introduced as a further goal of intergenerational solidarity, as explicitly demanded by the World Commission on Environment and Development (WCED, 1988). The concept of intragenerational equity is concerned with the fair distribution of the costs and benefits of dairy farming among social actors, whereas intergenerational solidarity implies the protection – and even the enhancement – of the rights and opportunities of future generations to benefit from the resources in use today.

The basic goals of sustainability in agriculture are environmental health, economic profitability and social equity. Sustainable livestock-farming practices should therefore be implemented at local scale, through a clear understanding of the way that agroecosystems work. But at the same time they
must take into account global issues and future considerations: global problems require global and local solutions; local problems should be solved at local level (Van Passel and Meul, 2012).

2. **GLOBAL CHANGING AND FARM RESILIENCE**

Global change is increasingly affecting agricultural production and threatening food security. Agriculture is highly exposed to climate change, which may have an impact on yields, location of production or costs of production, with potential risks for food supply, agricultural product prices and farm income (EUROSTAT, 2011). Furthermore, the extent to which the agriculture is developed is continuously affecting the environment through its direct impacts on land cover and ecosystems, global and regional cycles of carbon, nutrients and water (EEA, 2006).

The future sustainable dairy farming systems should pass for a long running transition to use innovative production methods and emission reduction measures (Foley et al., 2011). But intervention programs are difficult to set since links between the natural environment and farming practices are complex (EUROSTAT, 2011). Pressures and benefits from different types of agriculture confront the potentiability of intervention programs towards more sustainable agriculture systems. The dilemma is based on the fulfillment of different aspects of sustainability by different agricultural scenarios. In one side, extensification would benefit semi-natural habitats and reduce local pressures on soil, water and air but increase the area needed for agricultural production. In contrast, intensification would achieve the opposite but increases yields and input efficiency and would help to avoid further deforestation. It can nevertheless harm the environment if it is not properly managed and lead towards the homogenization of environmental conditions and further increasing pollution and disturbance of the nutrient cycle. Thus, the overall situation might still deteriorate (EAA, 2006).

The balance between intensive and extensive farming practices of grasslands can be roughly assessed by studying livestock densities. High stocking densities generally involve a risk of nutrient pollution and overgrazing, and a need to import animal feedstuffs. They are likely to contribute with more greenhouse gas emissions, as a result of manure production and enteric fermentation, and may also result in nutrient leaching into the water and air. At the other end of the range, a low level of livestock densities may increase the need for industrial fertilisers to be used on
agricultural land or lead to the risk of land abandonment resulting in the loss of environmental diversity (EUROSTAT, 2011).

In the context of the increasing demand for environmental considerations and climate change mitigation, the scenario expected to be the mainstream strategy of future organic dairy production should be accounted. Precision farming and organic practices, combining crop rotation and non-chemical crop protection, could increase overall efficiency in terms of land take, water use and nutrient management. Transforming the major food production areas in the world into low-input systems, however, would be a major operation, affecting productivity and food prices and potentially impacting global food security.

Down in the scale, fast changing environments should also consider the farm system. In fact, good farming practices in agricultural systems should regard their need for on-going adaptation to an ever-changing environment. Every farm is a complex system, characterized not only by the production method but also by a large number of farm and management specific aspects. The aims of a farm system under the resilience concept were to design a site-specific farm system such that, at all its levels (plant, field, and animals), would minimize the impact of sources of variation or disturbances, rather than ruling out the sources of variation (Napel et al., 2006). In this sense, they should offer solutions for buffering against climatic extremes, disease epidemics, changing nutrient availability, seasonal availability of forage and other stresses that will add to an already heterogeneous environmental condition (Goldringer et al., 2010). Under an adaptation model, these specific actions could be concretised in herd environment improvement (management, hygiene, feeding and husbandry) as the precondition to find an acceptable balance in limiting use of remedies but ensuring the necessary treatments in the real challenging of farmers and their practicing veterinarians.

3. ORGANIC AND LOW-INPUT FARMING SYSTEMS

Organic and low-input farming systems emerge as a future more resilient agriculture and a way to treat problems of food production and biodiversity as one. Organic food and farming systems address, both holistically and practically, many of the European policies on the sustainable management of natural resources, the safeguarding of biodiversity and landscapes, environmental concerns and improved integration of welfare friendly livestock systems in crop rotations and agroeocystems (Niggli et al., 2008).
Organic farming is a value-based method of agricultural production. The values are held in four principles that were articulated by the International Federation of Organic Movements (IFOAM). Through a worldwide participatory stakeholder process the values from the pioneers in organic agriculture were moved to the present time of globalization and extended growth of the organic sector (Luttikholt, 2007).

Success of organic farming is highly depending on the biodiversity used in its complex management system and adaptation to this fast changing world. Dairy farming under the rules of organic farming will always be land-based due to the requirement that manure production should not exceed 170 kg of nitrogen per year/ hectare of agricultural area. It implies a maximum stocking density of two dairy cows per hectare. With the existing standards, organic dairy farming can be applied in a structure of mixed as well as of specialised farming. However, various directions of intensification as seen in regular animal production are not possible because of the four principle standards. But a trend for a better economy is emerging regarding specialization and increase of scale (within given standards). De Wit and Verhoog (2007) pointed to the risk of conventionalization of organic agriculture with such trend, especially on the Principles of Ecology and Health. The use of off-farm inputs is an important factor in conventionalization itself and has negative effects of the core organic values.

4. GREENHOUSE GASES EMISSIONS AND DAIRYING

The main atmospheric gases associated with the ‘greenhouse effect’ are carbon dioxide (CO$_2$), chlorofluorocarbons (CFC’s), methane (CH$_4$) and nitrous oxide (N$_2$O). Greenhouse gases (GHG) arise from Agriculture, directly produced by livestock populations as well as forage together with feed production processes. Emissions of CO$_2$, CH$_4$ and N$_2$O are all influenced in an indirect way by intensive production systems (CEAS, 2000). Emissions of CH$_4$ result from human activities, such as agriculture, and the production and distribution of oil and gas.

In general, dairying in the EU is becoming more intensive and more specialised (CEAS, 2000). The dairy sector is aware of its high impact on the atmosphere, which arises from de-nitrification, the production of CH$_4$, ammonia (NH$_3$) volatilisation and CO$_2$. Furthermore, it also involves emissions to the wider environment of nutrients, heavy metals, and drug
residues such as antibiotics or hormones (Steinfeld et al., 2010; Lesschen et al., 2011).

For GHG emissions, the dairy farm itself (excluding the non-farming practices like dairy processing and packaging production) contributes to more than 80% of the impact for milk, from which about 50% is CH₄ from enteric fermentation, followed by N₂O from manure management and nitrous fertilizers. The third component, CO₂ is associated to the use of motors and electricity production.

Proportion between the different components in GHG emissions at farm level vary with the system. If methane generation per animal tends to be higher in low input systems than in those using feed supplements, ammonia emissions are highest in conventional, intensively managed systems (these occur during manure storage and application to arable and grassland). In terms of CO₂ and N₂O emissions, dairy production has only an indirect impact (mainly the use of energy to manufacture feed concentrates and to assist forage production as well as housing systems).

Organic farming may offer environmental protection and benefits through the less intensive use of land and by providing a changed balance in the use of inputs. Despite composting, there are no evidences suggesting losses of methane and ammonia are different from conventional farms (IDF, 2009). Thus, it remains the need to adopt management practices and technologies that improve productive efficiency to meet increasing product demand while minimizing the environmental impact of dairy production. In general, there are three possible strategies to follow: decrease of energy input; increase of energy efficiency; and, recycle the energy through biogas production (see de Haan et al., 1997).

Reducing the relatively high energy input in dairy systems would contribute to the global goal of reducing the concentration of CO₂ in the atmosphere (Capper et al., 2009). Garnsworthy (2004) found that a high-milk-producing system with high health and fertility status offers scope with minimizing CH₄ emissions per kilogram of milk, and that this can be achieved by reducing the number of milking herd replacements and the calving interval length, while increasing the average daily milk yield of the herd. Better management of slurry and manure involve high capital input to make structural improvements that will reduce impacts on soil, water and air (CEAS, 2000).

It is not easy to conclude which system has less impact on the environment. Organic farming does not seem to be better regarding Global Warming Potential due to the nature of the fodder and the lower productivity
of cows compared to conventional systems. The type of diets used for production of organic milk emits a little bit more of methane but there is a wide variation among studies (IDF, 2009). Regarding the energy use, since fodder is largely produced on-farm and the use of concentrated feed is limited, the non-renewable energy used is thus mainly located on-farm. Therefore, organic dairying generally consumes less energy than conventional farming. This is mainly due to differences in diet of the cows and the origin of fodder, as well of the lower use of energy consuming from mineral fertilizer.

The acidification is another damage indicator to take into account. The emission of acidifying substances per hectare are higher for conventional than for organic farming, although the acidification potential for 1 kg of organic milk is higher than for the conventional milk. This is mainly due to the higher milk yield (kg milk/hectare) in conventional farms. Large quantities of acidifying substances is small areas occurs at the intensive farming, leading into high concentrations in the environment that consequently damage the ecosystem.

There is no clear overall recommendation to be made regarding organic farming. Research is being conducted on whether to alter cow’s diet to decrease methane. Life-cycle assessment (LCA) methodology reflects present research priorities and is related to future legislation and labelling. For that, newly development impact assessment (water use, land occupation, ecotoxicology, pressure on biodiversity) are needed to constitute the base of initiatives to reduce the environment impact of the dairy industry that urge to be adopted by the necessary stakeholders (IDF, 2009). The farmers would ensure higher prices by becoming actively involved in other operations forming part of the value chain, such as distribution and/or small-scale processing.

5. MANAGEMENT AND FEEDING REGIMES

In organic and low-input systems, it is widely assumed that forage should be the primary source of nutrients for ruminants (Lund, 2006). Under the new legislation, the entire organic diet complies with the husbandry conditions that are expected to improve animal health and welfare (Sundrum, 2001). Which, however, are not ensured by the standards associated with organic farming per se (Vaarst et al., 2006). They have to be tested and if a problem is identified, it has to be solved both for reasons of health and welfare (Link, 2006).
In grazing systems, reducing stocking density may have a positive impact on dairy cow welfare (Kondo et al., 1989). High stocking densities affect the cow’s ability to access feeders and lying areas, although housing design also affects aggression and access to these resources (Niggli et al., 2008).

Under extensive grazing systems, cattle will often synchronize their behavior; that is, many animals in the group will feed, ruminate, and rest at the same time (Rook and Huckle, 1995). However the synchronization of behaviors may be reduced when cattle are housed intensively indoors perhaps because of competition for space or resources. Thus a trend exists now for housing dairy cows in freestall barns instead of tiestall barns. Consequently, cows are no longer fed individually; instead, they are fed as a group with a total mixed ration (TMR), where forages and concentrates are mixed together, allowing the producers to incorporate all the required ingredients into a single ration to meet cows’ nutritional requirements and optimize herd milk production. However, feeding cows as groups is more challenging because they can potentially sort feed and individual preferences can lead to cows eating different diets. In addition, social interactions among cows at the feed bunk can affect individual cowsteractions s is more challenging becaCurrent industry recommendations typically advise that each cow have approximately 0.6 m of linear feed bunk space to ensure that all cows can feed simultaneously (Grant and Albright, 2001). In addition, type and design of the feed barrier was shown to influence aggressive behavior between cows while they were eating (Huzzey et al., 2006). Several management and environmental factors affect feeding behavior of dairy cows. Increasing feed bunk space per cow may result in a reduction in displacements of subordinate cows by dominant cows while eating, in a reduced aggressive behavior among cows and improved feeding time (DeVries et al., 2004). In contrast, an increase in feeding competition may reduce intake and increase feeding rate, possibly increasing the risk for metabolic problems such as left displaced abomasums and subacute ruminal acidosis.

Meeting Increased Demands of Dairy Cattle under Extensive Systems

In the EU, dairy production with pasture or rangeland as the primary feed resource is mostly restricted to specific locations and linked with values like landscape conservation or rangeland management. All year grazing does occur in the EU. For example, in the Azores Islands more than 100,000 dairy cows
pass the day in pasture, year-round, with some concentrate supplementation when indoors or at milking. On the other hand, there are locations where pasture does not provide sufficient feed resource for dairy cows.

In organic and low-input dairy systems, the cow must graze grassland (outdoors) for a considerable period of time during the year. Such production system is associated with pasture rotations and different agronomic practices, which need to be coupled with strategies for pasture conservation and supplementation, as well as stocking rates.

Pasturing of dairy cows during summer is very common in most of the EU countries, even in the circumstances of intensive farming. But several factors limit the time that the cows are outside and can graze. Firstly, cows may need supplementary feeding indoors, to balance their N-rich grass diet and to provide them with extra energy. Secondly, the growing herd size at a farm may exceed the required area of grassland available for grazing of all cows, and supplementary feeding indoors is necessary anyway. Thirdly, mineral legislation may prohibit the farmer to put as many cows in the field as the farmer otherwise would do. Fourthly, farmers who apply automatic milking may prefer keeping their cows inside due to management reasons: for optimal timing of the individual cow (Ketelaar-de Lauwere et al., 1999).

In contrast to intensive systems, in extensive production systems the return per unit of input is relatively low. In dairy farming production is linked to the genetic disposition of the cows deployed for milk production and the amount and quality of feed that is fed. Grazing systems are typically based on maximal grazing of the animals during the year, as long as climate and growing conditions permit. For the remainder period, conserved feed needs to be available. During grazing periods in summer cows should have access to sufficient water supply and adequate shelter from sun, wind and rain.

Open discussion may deal with the issue that cows can also benefit from conditions provided indoors, most notably access to a high-quality diet and protection from environmental extremes (e.g., heat, cold, and wetness). If both pasture and indoor housing were provided freely, dairy cows would spend more time outdoors (summer) and preferred to lie down outdoors in summer nights and indoors in winter nights, at least under Danish conditions (Krohn et al., 1992). Unfortunately, the analysis of daily variation in weather has not been studied so far. Seasonal, pasture based systems of milk production will be of crucial importance in the future of sustainable dairy farming. If managed adequately, these systems guarantee a highly efficient and environmentally
friendly conversion of forage into milk, low use of concentrate supplementation, high standards of animal welfare and elevated consumers’ acceptance.

6. **ANIMAL HEALTH AND WELFARE CONCEPTS ON ORGANIC DAIRYING**

The regional intensification of animal production has led to more risks of environmental damage and to increased risks for animal and human health. In some extent, the regional agglomeration stimulated the development of vaccines, antibiotics, new technologies and herd health programmes in response (Steinfeld et al., 2010). In fact, diseases and outbreaks and zoonotic threats are more present and environmentally affected. Simultaneously, the risks for lower economic gains and lesser genetic diversity do exist.

To improve the sustainability of dairy production it is essential to establish practices to reduce medicine use, whilst safeguarding or improving herd health and productivity. Thus, the reduction in the use of chemical and the improvement of animal welfare is a common wish to reduce medication in organic animal husbandry (Vaarst et al., 2001). Under organic farming regulations, many of the conventional medical prophylactic measures associated with animal health control are either prohibited or restricted. The EC Regulation (2092/91 or 505/2012) on organic production specifies that disease prevention should be based on management systems that promote resistance to disease and recovery from infection, adapting the requirements to each species.

By consequence, animal health has been identified as a priority area for organic research. Previous studies on the animal health status of organic dairy cows have primarily focussed on areas where organic regulations may have most impact (Rutherford et al., 2009). Difficulties in methodology appeared when comparing organic and conventional farms. Comparative studies were mostly based on official health records where good records are available only for the most easily diagnosed diseases (Valle et al., 2007); and make production system comparisons will be also dependent of the health indicators in use. Furthermore, potential different treatment schemes by production system might happen.

Over time, questions regarding health in organic farms have moved beyond the focus under a systemic approach. There is a requirement to assess
current organic methods of animal health care and to identify practical constraints and critical points in their application.

Studies of health and health handling on organic dairy farms are scarce. It seems that main problems in descending order are: mastitis, fertility disorders, and hoof diseases whereas metabolic disorders such as acetonemia and milk fever are less frequent (Krutzinna et al., 1996). The role for the immune system in the incidence of such diseases is not truly highlighted but it is currently accepted that organic farming promotes the development of a more competent immune function, particularly if selection for disease resistance is implemented on animal selection.

There is only a limited amount of research in the area, but the reduction in the use of chemically allopathic treatments is part of the leading ideas in organic livestock production. Within organic dairy farming, there is a great interest on preventive strategies, earlier detection of diseases and treatments of diseased animals at an early stage. The implementation of these steps is expected to result in more favourable clinical outcomes and less antibiotic usage.

The majority of antimicrobial drugs are used for udder treatments (during lactation and combined with drying off; Menéndez Gonzalez et al., 2010), since mastitis is one of the most frequent health problems in dairy cattle, causing heavy economic losses in conventional dairying (Aeberhardt et al., 1997; Stärk et al., 1997). Advances made on this field are also of interest to reduce mastitis incidence as it represents more than two-thirds of antibiotic use in organic farms (Wagenaar et al., 2011), in particular if Holstein genetics (which is highly predisposed to clinical mastitis) is used. Also, the negative reflexes of udder diseases over the herd economy are relatively higher in organic than in conventional systems.

Again, based on the organic principles the organic dairy production should promote cow health and welfare. In this context, the use of robust cows well adapted to the organic farm is pursuit. Cow robustness can be defined as the cow’s ability to function well within a particular system, and this is reflected by its longevity. So, the longevity is a reflection of the cow’s ability to avoid being culled.

Resulting from its standards organic farming may have a positive impact on animal welfare, as it includes a broad range of practices complying with maintaince of healthy livestock under the high standards of welfare demands.

Recent interest in farm animal welfare focused more on pain or distress experienced by animals under “unnatural” conditions, with limited space and limitations on the full expression of natural behaviour, including social
interactions (Weary and von Keyserlingk, 2009), even if animal welfare goes beyond such concept. Combating overpopulation and ensuring a minimum of animal load per area, along with the imposition for grazing for a minimal period over the year, organic dairy systems provide a number of welfare benefits: cows have access to a more natural environment, they can perform behaviors that may be important to them such as grazing (Krohn, 1994), and cows on pasture sometimes experience a lower incidence of diseases such as mastitis (Washburn et al., 2002) and lameness (Hernandez-Mendo et al., 2007).

Nevertheless, cows in pasture-based systems can still suffer from poor welfare. Furthermore, non-regulated aspects of organic farming, such as the accepted ways of feeding and management, could restrict aspects of genetic selection towards a more “sustainable” biotype. Assessments of welfare on farm ought to include measures of the system and measures of how the system affects the animals (Rushen and de Passillé, 1998).

The assumption that grass-based systems have an advantage over other systems simply because cows are better adjusted to grazing needs to be confirmed. In fact, they can still suffer from poor welfare. Furthermore, animal biotype and genetics would solely generate a great variation under grazing conditions. The majority of the existing research comparing the welfare of dairy cow indoors and at pasture has focused on single indicators of animal welfare (e.g. behaviour, O’Connell, 1991) or health (e.g. mastitis, Washburn et al., 2002). It is mandatory to use a wide range of indicators simultaneously for a comprehensive evaluation of a production system (e.g. Broom et al., 1995). A wider and more complete evaluation of the cow’s welfare in organic farming is needed, and should include the animal behaviour, health (including mastitis and lameness), fertility/reproductive performance and measures of immune function.

Conventional dairy cows have been genetically bred to maximise the conversion of nutrients to milk. Prioritizing the nutrient for milk production reduces its availability to other biological functions such as maintenance of body weight, reproduction and health (Hoekstra et al., 1994; Pryce and Veerkamp, 2001) and increase the predisposition to inferior hoof health and lameness (Emanuelson, 1998). Maximizing DMI to overcome such deep negative energy balance (NEB) is one of the key issues in dairy cow feeding around calving and during early and mid lactation. NEB is specially exacerbated during the ‘Transition Period’ (three weeks prior to and three weeks after parturition; Grummer, 1995), coinciding to the period when most infectious diseases (e.g. mastitis) and metabolic disorders (e.g. milk fever,
ketosis, retained foetal membranes, metritis) occur (Goff and Horst, 1997; Mallard et al., 1998). Thus conventional dairying systems require feeding early lactating cows with balanced diets which offer excellent quality and palatability and simultaneously meet energy requirements and provide sufficient amounts of fiber in order to avoid subclinical metabolic diseases. This is currently the greatest challenge in dairy cow management.

Compared to cows under conventional management, cows in organic farms exhibit less negative energy balance or at least less body fat mobilization at early lactation (Fall et al., 2008), as demonstrated through the assessment of blood metabolites in cows under organic and conventional management and of cow’s body condition score (BCS) (Roesch et al., 2005; Fall et al., 2008). The resemblances in breeds and the overall management used in both systems, apart from the nutritional and sanitary impositions, could in part explain these findings. Such findings would indirectly influence the incidence of metabolic disorders, which may be lower in organic farms than in the conventional ones, if the cow genetics is taken into consideration (Fall, 2009). However, reports on this matter are controversial, and additional clarification is needed for breed influences on the incidence of metabolic disorders in organic dairy farms, by using new predictors for the occurrence of such diseases.

Adequate provision of suitable feed is one of the primary requirements to ensure the health of livestock (Lund, 2006; Manteca et al., 2008). Restrictions on the use of concentrates diets, according to organic standards (EEC 505/2012) may lead to a situation where organic dairy cows have difficulty in consuming enough energy. Recent studies on the levels of blood metabolites (β-hydroxybutyrate, free fat acids and insulin) before and after the change of legislation showed that blood changes were similar and that the incidence of clinical ketosis was not associated with herd type or the change of legislation. Thus the legislated changes in the nutritional management did not appear to have had any detrimental effects on the metabolic profiles of organic cows in early lactation and there was no evidence that organic cows were metabolically more challenged or had a severe negative energy balance (Blanco-Penedo et al., 2012).

Another source for the production stress includes the extreme environmental conditions that negatively impact the animal production, behavior, health and welfare. Climatic factors such as air temperature, solar radiation, relative humidity, airflow and their interactions, often limit animal performance in conventional dairying systems (Sharma et al., 1983). Dairy cattle research has tended to concentrate on genetic improvements to increase
milk production and on nutrient supply to the cow during early lactation. Little attention has been paid to the thermoregulatory ability of the modern cow as her capacity to produce milk has increased (Kadzere et al., 2002). However, most studies conducted in controlled environments focus on the effects of heat (temperatures between 24°C and environments: the modern-based systems where the animals are exposed to direct sunlight (Fuquay, 1981), rather than those of the cold (Hemsworth et al., 1995). Extensive data on the effect of heat stress on dairy production responses exists (Beede and Collier, 1986) and it has been demonstrated that milk production is reduced 15%, accompanied by a 35% decrease in the efficiency of energy utilization for productive purposes, when a lactating Holstein cow is transferred from an air temperature of 18 to 30°C (Beede and Collier, 1976). At ambient temperatures above 26°C, the cow reaches a point where she can no longer cool herself adequately and enters heat stress (Roenfeldt, 1998). High-producing cows are affected more than low-producing cows, particularly in early lactation, because the zone of thermal neutrality shifts to lower temperatures as milk production, feed intake, and metabolic heat production increase (Coppock et al., 1982), which may lead to rectal temperatures exceeding 39°C. At temperatures above 26°C, the cow reaches a state where she cannot cool herself adequately and enters heat stress (Roenfeldt, 1998). Heat stress is characterized by elevated respiration rates and rectal temperatures, and has been implicated in impaired metabolism (Bandaranayaka and Holmes, 1976) and in poor reproductive performance (Ingraham et al., 1974) in dairy cattle under conventional systems, independently of feed intake effects. Yet, Sharma et al. (1983) showed that different breeds have distinct ability to deal with high environmental temperatures, the called resistance to heat stress, thus allowing the dairyman to select the most suitable animal for a particular environment.

In organic and low-input systems, access to grassland should be complemented with several management measures to reduce the detrimental effects of heat stress and simultaneously improve animal welfare and milk yield. Some easily implemented measures include: the existence of shadows, either natural (trees) or artificial (portable shade-cloth blocking 50% of radiation or permanent structures), in size and amount adequate to allow all the cows to lie down, the availability of clean water and high quality forage within the shadowed areas, to minimizing the permanence indoors during milking, the use of sprinklers to cool housing facilities and to reduce insects infestation, as well as changings on the feed schedules, if necessary.
7. **Breeding for Organic and Low-Input Farming Systems**

Sustainability applied to dairy farming and organic dairy production has received increased attention in Europe, where pasture based milk production systems exists as alternative to conventional dairy farming, following the spirit of “naturalness”, to produce according to the principals of healthy and animal-friendly systems. Although organic and sustainable farming are not overlapping concepts, despite their aspirations for a production system based on good animal health and welfare, we can learn from experience from organic dairy farms that are closer to the sustainability-concept than the intensive farms do.

In sustainable and organic dairy systems the breed in use, the milk production and the reproductive management (with or without calving season) vary, hence inducing differences between dairy farms from different geographic location. These, combined to the cow biotype and milk yield, may determine the establishment of different goals for sustainable dairying diverging from those of conventional systems. And for that dairy cow breeding needs to be adapted towards an increased importance of fertility and other fitness traits, resulting in animals more suitable for organic, low-input systems. So, one important aspect in organic and low-input dairy farming is to define the cow attributes (the topic will be addressed later on).

A parameter of major influence for the reproductive traits is the breeding program and the strategies used in farms. Biological, ethical and economic considerations must be pondered when discussing future challenges for breeding in sustainable systems. On what concerns the reproductive management, in low-input systems and particularly in organic farming natural mating is preferred over artificial insemination (Nauta, 2010), while more sophisticated reproductive technologies such as embryo transfer are forbidden or discouraged. Also hormone treatments using GnRH agonist, progestagens or prostaglandins are prohibited, unless for treatment of medical conditions such as ovarian cysts or luteolysis. Thus most estrus synchronization programs are not an available strategy to modulate the reproductive efficiency in those farming systems (Garmo et al., 2010). Organic dairy herds using a bull in natural mating services usually have better fertility performance than those using the artificial insemination (Ahlman, 2010).

It is important to remember that by selecting the male line to be used for breeding, and while establishing the specific traits that are important in their
particular herd, the farmer strongly influences the daughters’ performance. Furthermore, the inappropriate use of sires may lead to a decline in the farm reproduction success (Rodriguez-Martinez et al., 2008.) Yet, for most countries, information concerning important traits for organic and low-input systems is often absent from the bull core information from a semen catalogue, limiting the identification of the conventional bulls best suited for organic production (Ahlman, 2010). Despite that total merit indexes adjusted for organic production developed in Switzerland, Germany and Canada (Bapst, 2001; Krogmeier, 2003; Rozzi et al., 2007), its use is somehow limited.

**Which Is the Expectable Reproductive Performance in Sustainable Dairying?**

In sustainable and organic dairy farming scenarios, functional and reproductive traits rather than milk yield are of key importance. The fertility of the cow is a major factor on the economy of the system (Figure 1) and is determined by multiple factors, including the management regime, environment, genetics, nutrition, and biological and health status (Löf, 2012).

![Figure 1. Major influences over cattle fertility in organic farming.](image)
Available information concerning reproductive efficiency and the productive performance for such systems is still limited. Currently, established reproductive management in sustainable dairying does not differ much from the conventional one. This is particularly true if the organic or low-input farm derived from a previously existent conventional farm (Nauta et al., 2006b). Reproductive efficiency of the cows is essential when establishing sustainable breeding programs and choosing the most suitable breed for a particular environment or management system (Ahlman, 2010).

As for the more conventional dairy systems, reproductive performance will be directed by the triangle energy balance/nutrition/milk yield in a breed-related manner. The breed will determine the feed efficiency and the equilibrium in the nutrient partition between the different organic compartments, thus influencing also the potential milk production level. It is the ability to mobilize body reserves at the onset of lactation, which was highly developed in modern Holstein-Friesian cows, that ultimately defines the cow's potential of milk production. One should remember, however, that energy density tends to be lower in organic feed rations (Reksen et al., 1999) and in grassland based systems, and low energy intake is known to influence the fertility in Holstein cows (Pryce et al., 1999; Rodriguez-Martinez et al., 2008).

Milk production as well as the ability to mobilize fat reserves and change the body energy metabolism (which can be followed through blood metabolites measurements) to assist milk production after calving differs with the breed genetic merit for milk yield (Barth et al., 2011), and rearing animals in low-input, grassland-based systems may highlight such differences. In addition, within the Holstein genetics, animals with similar genetic merit for milk production now show a wide range of genetic merit for fertility traits (from good - Fert⁺ to poor - Fert⁻), which has been associated to different priorities in the nutrition partition nutrients towards reproduction and milk production (Cummins et al., 2012).

Organic or low-input systems must comply with this framework and try to adjust the cow biotype in order to achieve the best profit from the system. The first parameter that needs to be defined is the existence of seasonal calving periods (block calving), like it happens in the New Zealand system (Kelly et al., 2004), or if calvings are allowed to occur year-round. This managerial decision may interfere with the breed selection and with forage and pastures management, as well as with milk availability, and indirectly may influence the reproductive parameters.
Animal selection for both functional and productive traits is complicated unless main objectives are clearly established. Functional traits in sustainable dairy farming may be grouped into three segments. One includes the reproductive parameters that positively correlate with a profitable system, such as the interval from calving to conception, the calving intervals, the age at first calving, the day open and the rate of stillbirth. Another important segment is the animal health and its resistance to diseases, in particular mastitis and parasitic infestations. The third element of the functional traits is constituted by parameters related to cow longevity, such as the cow robustness, including the leg and claws fitness, the weight gain and metabolism, the live weigh, and the productive lifespan (Figure 2).

Organic and low-input farms in different regions or countries have different basic conditions and different cow number and breeds, and may be subjected to different regulations, which must be taken into consideration when analyzing the available information on reproductive efficiency of the system. In Nordic countries, the organic dairy production usually presents a slightly higher herd size while the milk production level tends to be 10-30% lower than in conventional system (Reksen, 2005; Fall, 2009; Ahlman, 2010). However, the breeds used (mainly the Swedish Holstein and the Swedish or Norwegian Reds and their crosses) are similar. For this scenario, the overall fertility is slightly better for the organic farms, together with lower culling rates and longer longevity, than for the conventional ones (Ahlman, 2010; Löf, 2012), similarly to the reported for other countries and different cows breeds (Hovi et al., 2002).
Yet, this will probably change according to cow’s milk yield and with the cow’s breed. In fact, Reksen and colleagues (1999) working with Norwegian Red cows reported no differences in the reproductive indices in organic dairying in Norway, except for a reduction in the number of days open for organic cows, and pointed out the existence of predictors of compromised fertility, particularly when associated with external factors such as the calving season (summer vs. winter), the age of the cow (first calving intervals being longer in the organic than in conventional systems) and parity, which the authors associate to different individual ability to correct the negative energy metabolism balance with the forage between both systems. The breeding regime (natural services vs. artificial insemination) was shown to interfere with the calving interval in organic cows (Garmo et al., 2010), the interval to first and last artificial inseminations, as well as a reduction in the total number of inseminations (Löf et al., 2007; Ahlman, 2010) along with to increase in the number of cows getting pregnant at first insemination, even if highly influenced by the breed used in those farms (Ahlman et al., 2012).

When analysing the possible influence of individual and environmental factors (highlighting the Genetic x Environmental interaction) over the reproductive efficiency in organic farms, one can conclude that there is room for improvement within this production system.

Moreover, it is generally accepted that lower intensity of production will ease the stress pressure over the cow under organic systems thus improving its ability to survive longer and have an increased productive lifespan (Reksen et al., 1999; Fall, 2009; Ahlman, 2010).

Thus, on what concerns to the reproductive management, the sustainable dairymen must ponder on the use of alternative breeding scenarios (Nauta, 2009), always taking into account the interaction GxE and the weighing of different traits targeted in the managerial outcome proposed for the farm:

1. The use of adapted conventional breeding, where the reproduction techniques used should also be addressed, e.g. by excluding direct use of ET bulls.
2. The use of separate breeding programs within the organic production system, where bulls are evaluated based on their daughters’ performance in organic farms. All modern reproduction techniques except AI should be excluded.
3. Use local breeding programs based on natural mating within the farm or region.
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8. SELECTING THE BEST COW BIOTYPE FOR LOW-INPUT SYSTEMS

The selection of the most suitable animal or breed to a particular farm system, whatever the production involved, might represent one major factor of success. Dairy producers need to understand that the final production does reflect both the action of the animal genes and the environment (Nauta et al., 2006a), although the later might be versatile. Thus, expectably not all the cattle breeds behave as one, depending on the production environment.

It is frequently discussed that cow performance frequently differs between organic and conventional herds (Pryce et al., 2004), hence raising the question of whether the dairy cows have the ability to adapt to the organic production environment. Several concerns were raised regarding the ability of high producing breeds to adapt to organic environments, particularly to a reduction in the energy and protein intake and the limited use of antimicrobial drugs. The main issue relates to the Genotype vs. Environmental interactions, which were found to be of major importance in the occurrence of production traits (Nauta et al., 2006a). High-producing Holstein Frisian cows might not be the most suitable animal for sustainable dairy farming as it requires high standards of nutritional and management support. Those animals were mainly selected for high production in a highly controlled environment that differs from that of organic production, mainly regarding a more demanding feed quality, ration composition, feed management and feed intake, animal health management, and housing, although they are more efficient in the use of resources per unit of milk produced (Dobson, 2009).

A specific organic or sustainable breed of dairy aptitude doesn’t exist. Under controlled conditions for technology and drug usage, and the actual limitations concerning the feed origin, as it happens in organic farming, animals need to fit and cope with the environment. Selection of the animal biotype is important to reach a good balance between the production level, the animal physiology (welfare, behaviour, health, reproduction), and the societal value about the production system.

Therefore different breeds are needed for different local conditions, making the biodiversity of farm animals a necessity (Spengler Neff, 2007; Spengler Neff, 2009).

On the basis of the current knowledge on dairy cow adaptation to different environments it is suggested that organic farmers should use robust breeds with broad breeding goals, including both production and functional traits.
(Pryce et al., 2004). In sustainable/organic systems, which encompass with reduced intensity of production and moderate to low-input systems, the functional traits (usually of low heritability and often unfavorably correlated with milk production) such as health and longevity, and the milk quality, would be more important than the productive traits itselfs (with higher heritability). Also, selection of the animals would follow these guidelines, and increased value should be attributed to the functional traits, like the disease resistance (in particular to mastitis), parasite resistance, strong legs and claws, foraging ability, fertility traits, persistency of lactation and increased milk yield from first to third lactation and longevity (Bapst, 2001; Pryce et al., 2004; Ahlman et al., 2012; Spengler Neff, 2012a, b).

As a resume, the decision on what sort of animal that best suits a farm is a main issue when deciding for sustainable dairying. The foreseen biotype for sustainable dairy systems is a very balanced cow which is highly efficient at producing milk using less feed, i.e. a better feed-converter animal. There is no prescription ever for the best cow for a given region. An equilibrium have to be established in sustainable dairy systems between the cow biotype, feeding and land management, and a compromise with the milk yield must be reached taking into consideration the economic profit of the system. So, it is important to choose the breed most suited to a particular management system and to the market where it is intend to sell the milk, since the existence of specific fed requirements and the need to minimize the use of drugs to treat or prevent diseases will have reflexes on the cow productivity. Cross breeding with dual-purpose breeds may be another solution available to sustainable and organic dairy farmers (Nauta, 2009; Spengler Neff et al., 2012b), since it may allow improving milk yielding of local breeds, which are generally best adapted to local environmental conditions.

9. SOCIO-ECONOMIC ISSUES IN SUSTAINABLE DAIRY SYSTEMS

The most likely future scenarios suggest that the dairy sector will continue to bear at least part of the cost of global warming. As a result, at present the best bet might be to identify and quantify the consequences of climate change, and then work on identifying the best options for the future (Easterling et al., 2007; Thornton et al., 2007; Nardone et al., 2010).
In operational terms, it is essential that the end-users, their aims and motivations will be actively involved (Bélanger et al., 2012). Obviously, the farmers themselves are responsible for practical actions at farm level, and thus play a key role in social equity. The current loss of purchasing power among livestock producers has a direct impact on generational solidarity.

Other factors, such as the hard work involved and the lack of rural areas facilities, favour the disappearance of farms (Bernués et al., 2011), aggravating the imbalance in regard to urban areas. Research into intergenerational issues suggests that younger generations see no future for themselves in dairy farming (Perea et al., 2010).

If the social equity of dairy farms is to be guaranteed, there has to be a full understanding of the farmers’ view of sustainability, and priorities have to be established with a view to making the rural world more attractive, and providing it with improved facilities and amenities.

10. ON THE NEED FOR NEW POLITICAL CONTEXTS

There is a considerable lack of information regarding what scenarios are desirable for the dairy farms of the future. Some authors regard the link of sustainability evaluations and simulation models as a promising development (Oudshoorn et al., 2011; Vayssière et al., 2011). One major constraint for this approach is the limited availability of real-time data to base the design and monitoring of forecast scenarios. Moreover, sustainability-evaluation tools alone still do not generate the required quantitative information, so simulation models may serve as a robust framework for qualitative assessment. In this context, it is difficult to draw up general guidelines regarding which models are most appropriate, but there is a general agreement regarding a number of key criteria: sustainable dairy farm models should be diversified, robust, with efficient links to the industry. They should be more self-sufficient, place greater emphasis on direct marketing and have closer links to local lifestyles and fewer links to business (Steinfeld et al., 2006; Thornton et al., 2007; Gill et al., 2010; Hoffmann, 2010; Nardone et al. 2010; Bernués et al., 2011; ten Napel et al., 2011; Augustin et al., 2012).

The dairy industry will also have to bear the cost of relocation, since some current farm locations are very unlikely to be sustainable in future. Thus, simulation models might provide a framework for predictions regarding global warming, and can be used as a basic instrument for analysing the different options (Turnpenny et al., 2001).
The major critical issues in regards to climate change are those related to water, soil and habitats (Gill et al., 2010). Other relevant aspects are those linked to energy efficiency, both at farm level and in terms of more effective relationships with the industry (Augustin et al., 2012).

The best-placed dairy farms in the race towards sustainability will therefore be those most closely bound to the land, i.e. organic farms or integrated agriculture/livestock systems, whose efficiency – and not just productive efficiency – is today a priority.

Since sustainable production tends to be more expensive, due to lower physical yields and higher production costs, it is clearly essential to devise and implement a system of rewards for the efforts involved in sustainability at a farm level (Gibbon, 2005; Jay, 2007; Roeder et al., 2010). Clearly, the farmer is the first to benefit from sustainable livestock rearing, since it enables him to obtain profits without jeopardising the future production capacity, i.e. without losing natural or man-made capital. But it also benefits the Society, while having a positive impact on other related economic activities: it provides food and non-productive services, both environmental and social. The question is how much are those non-productive services worthwhile. If the market does not reward them through higher prices, society will have to do so. Otherwise, market inertia will rebalance the systems at a less sustainable, though economically fair, level. Society therefore has to assume part of the cost of sustainability, since it also profits of the benefits. This is crucial for the implementation of sustainable dairy farming.

The EU has pioneered the development of reward systems aimed to a greater degree of sustainability. However, a number of studies (e.g. Franco et al., 2012) highlighted failures in certain agricultural and rural development policies. A thorough review of this whole issue is both essential and controversial. The need for balanced reward systems must be weighed against the fact that they run counter to the current trend of global agreements on the gradual elimination of production subsidies and tariffs.

11. ASSESSING SUSTAINABILITY IN DAIRY SYSTEMS

The suitability, advantages and drawbacks of the various currently used methods to assess sustainability have been examined in a number of studies (Pannell and Glenn, 2000; Payraudeau and van der Werf, 2005; Singh et al., 2012). Presently, they are not sufficiently reliable to fully support decisions
First difficulties for the assessment of sustainability are inherent to the very nature of the issue: sustainability requires multidisciplinary conceptual frameworks in which social, economic and ecological considerations form a coherent whole. Failure to ensure adequate representation of all three dimensions often leads to fairly speculative multidimensional approaches using a range of tools. The social aspects of sustainability tend to be those least represented tools (Van Passel and Meul, 2012).

Methods for measuring sustainability face three major constraints: (1) sustainability cannot be directly measured; (2) there are no absolute scales for sustainability; (3) no quantitative criteria are available, so that it is impossible at present to predict with any certainty the extent to which changes made today will ensure sustainability tomorrow.

The first constraint gave rise to the development of the so-called sustainability indicators, which can be defined as the indirect measurements of sustainability. At farm level, clearly, the best approaches are those based directly on sets of indicators, whilst at higher levels a certain degree of aggregation is required. Various sets of indicators have been applied to dairy systems, including the IDEA method (Vilain, 2008), one of the first tools to be designed in Europe, the RISE method (Häni et al., 2003) in Switzerland, the FarmSmart in the UK (Tzilivakis and Lewins, 2004), and the MESMIS framework (Masera et al., 1999).

Since priorities for evaluation are different for each setting, the ideal approach is to develop a specific set of indicators (Hueting and Reijnders, 2004). In fact, when building the conceptual framework, two key questions need to be considered: which indicators should be selected? And what is the optimal value for each indicator in terms of sustainability? Since a cohort of researchers, end-users and experts selects the indicators and optimal values they inevitably reflect a certain degree of subjectivity, which also masks the effects of premises and simplifications. These problems have been widely examined by researchers (Table 1).

At political or industry level, arguments have been adduced in favour of compound indices, on the grounds that they are easier to use (Van Passel and Meul, 2012). Methods of aggregating indicators vary considerably, and also depend on whether the approach is ecological or economic.

Arguments have been advanced in favour of adopting an economic approach for evaluating dairy systems, based generally on the principles of productive efficiency and utility theory.
Table 1. Summary examples of sustainability indices

<table>
<thead>
<tr>
<th>Name</th>
<th>Number of sub-indicators</th>
<th>Scaling</th>
<th>Weighting</th>
<th>Aggregation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index of sustainable and economic welfare</td>
<td>20</td>
<td>Monetary terms</td>
<td>Equal</td>
<td>Arithmetic average</td>
</tr>
<tr>
<td>Index of environmental friendliness</td>
<td>11</td>
<td>Normalization</td>
<td>Subjective</td>
<td>Weighted sum</td>
</tr>
<tr>
<td>Ecological footprint</td>
<td>6</td>
<td>Area</td>
<td>Equal</td>
<td>Summation</td>
</tr>
<tr>
<td>Carbon footprint</td>
<td>4</td>
<td>Normalization</td>
<td>Equal</td>
<td>Summation</td>
</tr>
<tr>
<td>Environmental sustainability index</td>
<td>68</td>
<td>Normalization</td>
<td>Equal</td>
<td>Arithmetic average</td>
</tr>
<tr>
<td>Human development index</td>
<td>3</td>
<td>0 – 1, using minimum and maximum value for each indicator as goal post</td>
<td>Equal</td>
<td>Arithmetic average</td>
</tr>
<tr>
<td>Environmental vulnerability index</td>
<td>50</td>
<td>Aim = 7, worst = 7</td>
<td>Equal</td>
<td>Average</td>
</tr>
<tr>
<td>Well being index</td>
<td>87</td>
<td>0 - 100</td>
<td>Subjective</td>
<td>Weighted average</td>
</tr>
<tr>
<td>Live cycle index</td>
<td>21</td>
<td>Linear and non linear functions</td>
<td>AHP</td>
<td>Geometric mean</td>
</tr>
<tr>
<td>G score</td>
<td>5</td>
<td>Subjective</td>
<td>Equal</td>
<td>Summation</td>
</tr>
</tbody>
</table>

The value of resources and products are assumed to reflect expected utilities derived from the consumption or existence of the product (Tyteca, 1998; van Calker et al., 2006). This concept of value is the most readily-comprehensible and acceptable one for decision-makers with settings based on economic values (Farrell and Hart, 1998).

Models are more viable if they are able to reflect that certain forms of capital are irreplaceable, or that at least a minimum threshold level is required to avoid irreversible loss (Perman et al. 2003; Kuosmanen and Kuosmanen, 2009). Classical economic approaches are based on measures of efficiency use for the different forms of capital, or on cost-benefit analysis (Figge and Hahn, 2004a, 2005; Meul et al., 2005). In practical terms, these approaches are limited by the method used to assign economic values, since the market can only provide the direct utility of some elements of the model.
In general, these models have achieved a fair indication of whether or not a production unit is sustainable. However, few models have focused on the question of what is the most sustainable way of assigning resources. One such model is the sustainable-value method (Figge and Hahn, 2004b). Conceptually, it is based on the constant-capital rule, and is essentially a relative measure of efficiency. A number of methodological issues have recently been highlighted which need to be revised in order to ensure the proper implementation of the sustainable-value method (Kuosmanen and Kuosmanen, 2009). Even so, this remains a promising approach, which is likely to receive considerable attention in the forthcoming years.

**CONCLUSION**

This chapter book aims to present a comprehensive and up-to-date overview of the results of research and innovation in sustainable dairy farms. The dairy sector supports a long-term vision in which a top performance in sustainability is the goal. Based on this vision, ambitions have been formulated, which in many cases go much further than the current standards. Priority issues in sustainability are the following: animal welfare, a clean environment, climate neutral production, on-farm nature and biodiversity, close connection with society and consumers, healthy, and safe dairy products and no depletion of resources. All are essential and the balance and integration of these focus areas constructively contribute to the sustainability of this farm system.

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electricity. *Biomass Bioenerg* 31, 759


ci.


**ABSTRACT**

Early detection of mastitis with subclinical symptoms is possible by determining somatic cell count (SCC). SCC is the most widely accepted indicator of the mammary gland health as well as milk quality and its technological suitability. The authors’ research has revealed that an increase of SCC (independently of a breed of cows) mainly causes a rise in a total crude protein content and a distinct reduction in lactose level (P≤0.01). Moreover, SCC also lengthens the time of milk enzymatic coagulation (P≤0.01) but it does not influence its thermal stability. Distinct negative relationship between casein content and SCC is confirmed by relatively high value of correlation coefficient (r=−0.59). In
the authors’ studies the significant interactions (breed of cows x SCC) for the daily yield of cows, content of protein, casein and lactose, protein to fat ratio and rennet-induced milk coagulation time also have been stated, which indicates a differentiated response of various breeds of cows to udder inflammations. Holstein-Friesian cows are more sensitive to decline of daily yield, that is reflected in higher negative value of correlation coefficient between SCC and milk yield (-0.245). In Simmental and Jersey cows the correlations were negative as well but their values were substantially lower (r=-0.123 and r=-0.148) and statistically insignificant. With the age of cows increase in SCC was noted and in the cows of local breeds (Polish Red, Polish Black and White, Whitebacked) and Jersey that rise was much smaller in comparison to Polish Holstein-Friesian cows. Significant interaction (P≤0.05) for SCC between breed of cows and subsequent lactation was indicated. However, the significant changes in milk constituents were recorded only when the SCC exceeded 500 thous. ml\(^{-1}\), that is in milk that does not meet the current regulatory quality standards.

Somatic cell count also affects the changes in whey protein content. Rise of SCC decreased the content of major albumins, i.e. alpha-LA and beta-LG, by small degree, and that was confirmed by very low statistically insignificant correlation coefficients \((r=-0.07 \text{ i } r=-0.05)\). Negative value of both correlations, though, indicates a direction of changes and may imply that in more advanced stages of udder diseases the decrease of milk proteins is likely to be higher. However, with rise of SCC, content of immunoactive proteins (lactoferrin and lysozyme) as well as bovine albumin serum (BSA) significantly increased. The significant impact of SCC on content of these proteins in milk is confirmed by relatively high positive values of computed correlation coefficients (lactoferrin \(r=0.65\), lysozyme \(r=0.63\) and BSA \(r=0.59\)). In the case of BSA that correlations were clearly differentiated in particular breeds of cows, i.e. \(r=0.711\) for Holstein-Friesian, \(r=0.577\) for Simmental and \(r=0.472\) for Jersey. Thus, it can be assumed that there is a differentiated degree of permeability of mammary gland cell membranes in cows of various breeds.

1. Introduction

Mammary gland inflammations are induced by as many as even over 140 various types of microorganisms inhabiting both animal and its environment [Malinowski and Kłossowska, 2000]. The cause of mastitis can be a variety of microorganisms such as bacteria, mycoplasmas, yeast like fungi, algae and in the rare cases viruses [Bradley, 2002; Khan and Khan, 2006]. The principal
etiological factors inducing the inflammation of mammary gland of cows are bacteria. The severity of clinical symptoms and course of mammary gland inflammation is largely dependent on the type of bacteria causing the infection. With regard to the etiological factor, a classic division of mastitis into two categories, depending on a method of infection, was established, i.e. mammary gland inflammation caused by “infectious” factors (e.g. *Staphylococcus aureus*) and “environmental” (e.g. *Escherichia coli*). Microorganisms inducing the infectious mastitis exist on the skin of udder and they are well adapted to a survival and proliferation in host organism, in particular in the mammary gland. Nevertheless, they are not adjusted to life outside the body of a host. These bacteria are primarily responsible for subclinical and chronic inflammation of the mammary gland. The “environmental” factors, i.e. bacteria living in an environment of cows (bedding, water, earth), are less virulent pathogens and generally rapidly eradicated by the immune system of a host [Blowey and Edmondson, 1995; Bradley, 2002]. However, *Staphylococcus aureus* and *Streptococcus agalactiae* as well as the environmental bacteria, mainly from the *coli* group: *Escherichia coli* (most frequently) and *Klebsiella pneumoniae* perform a primary function in the inflammation induction [Smulski et al., 2011].

According to Klastrup et al. [1987], the susceptibility to mastitis was in 25% a result of environmental factors, 20% – genetic factors and 50% – herd management.

### 2. Somatic Cell Count as the Indicator of Mammary Gland Health Status

In numerous research it was shown that somatic cell count (SCC) in cow milk is a good indicator of mammary gland health status. Furthermore, occurring subclinical and clinical inflammations are associated with the significant increase in the count of somatic cells [Rainard and Riollet, 2006]. This fact was confirmed by generally high positive correlation coefficients between SCC and inflammation status \((r=0.30-0.97)\) [Carlen et al., 2004; Bloemhof et al., 2009].

It should be emphasized that interbreed differences in the susceptibility of cows to mastitis exist. Rupp and Boichard [2003] reported that cows of dairy breeds originating from the eastern France (Montbéliarde and Abondance) and central Europe (Simmental and Brown Swiss) are less frequent in clinical form.
of mastitis and their milk contains lower level of somatic cells compared to Holstein breed. Gołębiowski and Brzozowski [2007], who claimed that Montbéliarde breed characterizes a higher susceptibility to mastitis than Holstein, due to the lower by 23-38% somatic cell count in milk, also confirmed this fact.

The SCC of milk includes leucocytes derived from the blood (75%), i.e. neutrophils, macrophages, lymphocytes and erythrocytes, and epithelium cells of udder (25%). In milk obtained from a healthy udder leucocytes are consisted in 50±10% of neutrophils (polymorphonuclear granulocytes – PMNs), in 36±9% of lymphocytes and in 14±2% of macrophages. It should be noted that the percentage of particular types of cells varies depending on the udder health, age and stage of lactation. Share of leukocytes increase in response to bacterial infection, tissue injury, stress and final stadium of lactation. Leucocytes are transported into the milk gland directly from the blood as a response to the chemical substances, released by mammary glands during inflammation [Aniulis et al., 2003; Sharma et al., 2011]. The increase in somatic cell count in milk, primarily neutrophil granulocytes (neutrophils), is the first signal informing about changes in the health status of mammary gland. In uninfected quarters of mammary gland, i.e. without mastitis, SCC is lower than 150 thous. ml⁻¹, at simultaneously lower percentage of neutrophils (5-25% of the total SCC). It is assumed that the somatic cell count in cow milk exceeding 150 thous. ml⁻¹ is one of the first signals of mammary gland infection. During inflammation an elevation in milk SCC is mainly conditioned by the increase of share of the polymorphonuclear cells by 99-100% [Schukken et al., 2003; Tao and Mallard, 2007]. Scheppers et al. [1997] established the so called “physiological threshold level”, i.e. the SCC limit of 200 thous. ml⁻¹, to differentiate between a healthy and infected quarter. Furthermore, they stated that each doubling of SCC above 50 thous. ml⁻¹ results in losses in milk production. Griffin et al. [1987] established, however, the SCC lower limit of subclinical stage of mastitis at 125 thous. cells ml⁻¹, whereas the upper limit at 250 thous. cells ml⁻¹. Whereas Kherli and Shuster [1994] as a limit value of SCC 100 thous. ml⁻¹ and Harmon [1994] accepted only 50 thous. ml⁻¹.

For the dairy industry the most pernicious are subclinical (non-symptomatic) inflammations since milk obtained often becomes the commercial milk. In Europe, in accordance to the Commission Regulation (EC) No 1662/2006 of 6 November 2006 (No L230/4) modifying the Regulation (EC) No 853/2004 of the European Parliament and of the Council of 29 April 2004, milk intended for human consumption should not contain
Somatic Cell Count as the Factor Conditioning Productivity …

more than 400 thous. somatic cells ml\(^{-1}\). Furthermore, the identical limits apply
in Canada and New Zealand. In the U.S. the official standard was up to 750
thous. cells ml\(^{-1}\) until 2011, however, a gradual reduction of SCC limit to the
requirements of the European Union is planned, in order to allow the
American producers to export dairy products to European markets [National
 Mastitis Council, 2011].

3. **ECONOMIC EFFECTS OF UDDE INFLAMMATION**

Mastitis remains the most expensive and hard to overcome by veterinary
methods the disease affecting dairy cattle. According to SABRE [2006], the
total losses due to mastitis in the dairy industry in the EU in 2005 amounted to
EUR 1.55 billion.

Bovine mammary gland inflammations result not only in reduction of milk
synthesis but also in alterations in milk constituents, leading to deterioration of
its nutritive value and technological suitability. Simultaneously, they belong to
the most common causes (except for infertility and lameness) of culling of
cows from herd. The udder inflammations constitutes approximately 70% of
all economic farm losses [Huijps et al., 2008]. Costs associated with the
mammary gland inflammation differentiate depending on the etiological
factor, course of clinical form, animal and milk prices, fodders, services and
drugs [Halasa et al., 2007; Malinowski et al., 2011]. In various countries these
costs are varied, however, each time they are a large financial burden in dairy
farms (Table 1). For instance, in the United States in the case of one clinical
mastitis the costs are estimated at USD 107 and in Sweden even the USD 735
[Malinowski et al., 2011]. The size of losses associated with an occurrence of
mammary gland inflammations also results in a number of cows in herd and a
form of mastitis. Huijps et al. [2008] reported that in smaller herds, consisting
of approximately 30 dairy cows, losses were definitely lower (EUR 17 /cow
per year) in relation to the herds comprising of approximately 160 heads (EUR
198 /cow per year). In the extreme cases, at the presence of inflammations in
clinical form, the average losses were estimated at EUR 210, varying from
EUR 164 to EUR 235 /cow/month, respectively, in the final and the initial
stage of lactation. In the case of subclinical affliction in the herd of cows with
productivity of 8,500 kg/cow/305 days of lactation, at SCC in bulk tank milk
amounted to approximately 200 thous. ml\(^{-1}\), losses were estimated at EUR 20
/cow per year. Chassagne et al. [2005] and Aniulis et al. [2003] stated that the
most susceptible to mastitis were animals in the initial period of lactation.
Table 1. Costs bearing to treatment and prevention of mastitis according to various authors

<table>
<thead>
<tr>
<th>Cost</th>
<th>Mastitis status</th>
<th>Additional information</th>
<th>Country</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EUR 28 /cow/year</td>
<td>average cost</td>
<td>USA, Michigan</td>
<td>Kaneene and Hurd, 1990</td>
<td></td>
</tr>
<tr>
<td>EUR 31 /cow/year</td>
<td>average cost</td>
<td>USA, Ohio</td>
<td>Miller and Dorn, 1990</td>
<td></td>
</tr>
<tr>
<td>EUR 22 /cow/year</td>
<td>average cost</td>
<td>USA, California</td>
<td>Sischo et al., 1991</td>
<td></td>
</tr>
<tr>
<td>EUR 279 /cow/year</td>
<td>average cost in summer</td>
<td>England</td>
<td>Hillerton et al., 1992</td>
<td></td>
</tr>
<tr>
<td>EUR 102 /cow/year</td>
<td>subclinical</td>
<td>average cost</td>
<td>England</td>
<td>McInerney et al., 1992</td>
</tr>
<tr>
<td>EUR 20 /cow/year</td>
<td>average cost</td>
<td>Germany</td>
<td>Reinsch and Dempfle, 1997</td>
<td></td>
</tr>
<tr>
<td>EUR 287 /average case of cow/year</td>
<td>average cost</td>
<td>England</td>
<td>Kossaibati and Essleniant, 1997</td>
<td></td>
</tr>
<tr>
<td>EUR 26 (19-32) /cow/year</td>
<td>cost of control of udder health status because of mastitis</td>
<td>France</td>
<td>Fourichon et al., 2001</td>
<td></td>
</tr>
<tr>
<td>EUR 65-182 /cow/year</td>
<td>subclinical and clinical</td>
<td>the Netherlands</td>
<td>Huijps et al., 2008</td>
<td></td>
</tr>
<tr>
<td>EUR 117 /cow/lactation</td>
<td></td>
<td>Spain</td>
<td>Pérez-Cabal et al., 2008</td>
<td></td>
</tr>
<tr>
<td>EUR 71 /cow/year</td>
<td></td>
<td>USA, New York</td>
<td>Bar et al., 2008</td>
<td></td>
</tr>
<tr>
<td>EUR 179 /cow</td>
<td>cost of complete treatment</td>
<td>USA, New York</td>
<td>Bar et al., 2008</td>
<td></td>
</tr>
<tr>
<td>EUR 78 (17-198) /cow/year</td>
<td>in dependence on herd size, assessed by farmers</td>
<td>the Netherlands</td>
<td>Huijps et al., 2008</td>
<td></td>
</tr>
<tr>
<td>EUR 210 (164-235) /cow/month</td>
<td>clinical</td>
<td>cost in () respectively in the final and the initial stage of lactation</td>
<td>the Netherlands</td>
<td>Huijps et al., 2008</td>
</tr>
</tbody>
</table>
According to the French data [Chassagne et al., 2005], it results that in the first 30 days after calving, even in a well-managed herd, the percentage of those animals amounts to approximately 30%. This is due to an attenuation of immune defense reaction of cow in a perinatal period and more frequent incidence of mammary gland inflammation associated with that. According to the data provided by SABRE [2006], in the European Union 30% of cows remains infected by mastitis in 2005. Furthermore, the analyses conducted in the same period in Poland proved that the percentage of animals with symptoms of mastitis is at a similar level but clinical forms of the disease are recognized in 2-5% of cows [Głowacki, 2006]. The research conducted in Lithuania by Aniulis et al. [2003] demonstrated that about 42-47% of cows in

<table>
<thead>
<tr>
<th>Cost</th>
<th>Mastitis status</th>
<th>Additional information</th>
<th>Country</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUR 275 /cow/year</td>
<td>Clinical</td>
<td></td>
<td>Sweden</td>
<td>Nielsen, 2009</td>
</tr>
<tr>
<td>EUR 60 /cow/year</td>
<td>subclinical</td>
<td></td>
<td>Sweden</td>
<td>Nielsen, 2009</td>
</tr>
<tr>
<td>USD 224-275 /cow</td>
<td>clinical</td>
<td>average total cost of</td>
<td>the Netherlands and Canada</td>
<td>Steeneveld et al., 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>USD 107 (161.8-344.2) /cow</td>
<td>clinical</td>
<td></td>
<td>USA</td>
<td>Malinowski et al., 2011</td>
</tr>
<tr>
<td>USD 735 /cow</td>
<td>clinical</td>
<td>cost of treatment of</td>
<td>Sweden</td>
<td>Malinowski et al., 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>one case</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EGP* 15.80±5.25 – 1734±281 /cow/lactation</td>
<td>clinical</td>
<td>in dependence on SCC, respectively: ≤50 – &gt;2000 thous. cells ml(^{-1})</td>
<td>Egypt Friesian</td>
<td>El-Awady and Oudah, 2011</td>
</tr>
</tbody>
</table>

Prevention

<table>
<thead>
<tr>
<th>Cost</th>
<th>Mastitis status</th>
<th>Additional information</th>
<th>Country</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUR 3.56 (0-22) /cow/year</td>
<td></td>
<td></td>
<td>USA, Michigan</td>
<td>Kaneene and Hurd, 1990</td>
</tr>
<tr>
<td>EUR 4-12 /cow/year</td>
<td></td>
<td></td>
<td>USA, Ohio</td>
<td>Miller and Dorn, 1990</td>
</tr>
<tr>
<td>EUR 4 /cow/year</td>
<td></td>
<td></td>
<td>USA, California</td>
<td>Sischo et al., 1991</td>
</tr>
<tr>
<td>EUR 3 /cow/year</td>
<td></td>
<td></td>
<td>Germany</td>
<td>Reinsch and Dempfle, 1997</td>
</tr>
<tr>
<td>EUR 50 /cow/year</td>
<td></td>
<td></td>
<td>Sweden</td>
<td>Nielsen, 2009</td>
</tr>
</tbody>
</table>

*EGP – Egyptian pounds.
that country had symptoms of subclinical mastitis. It should be emphasized that subclinical states are more serious problem in dairy herds. Averagely approximately 40% of cows are constantly affected by changes specific to subclinical mammary gland inflammations [Malinowski and Kłossowska, 2000]. The changes are usually long-term and often unrecognized by farmer and ipso facto undiagnosed, especially in herds without SCC monitoring. According to Östensson et al. [2012], the degree of spreading of subclinical form of mastitis is very high, both on the level of quarter (63.2%) and cow (88.6%). However, these results are much higher than those obtained by other authors [Nam et al., 2010; Petrovski et al., 2011], i.e. 35% and 55%, respectively.

4. Effect of SCC on Productivity of Cows and Basic Composition of Milk

Susceptibility or resistance to mastitis of dairy cows is genetically conditioned. The genetic antagonism between milk yield and udder health status has been proved, resting on a deterioration of health status of mammary gland as a result of selection for increase in milk yield [Rupp and Boichard, 2003].

The disease is therefore a problem in high productive herds, in which an intensive husbandry conditions are used. However, direct selection for mastitis resistance is not widely applied because of its low heritability. The results of numerous research (Table 2 and 3) indicate that the values of heritability for somatic cell count (SCC) or natural logarithm of SCC (SCS – somatic cell score) are not exceeded 0.20. However, the results obtained by Dube et al. [2008] point to lower value of heritability in primiparous Jersey cows ($h^2=0.07$) and slightly higher at multiparous cows ($h^2=0.11$) (Table 3). The heritability estimates for udder inflammations are even lower than for SCC (Table 3). Low values of heritability for susceptibility of cows to mastitis do not mean, however, the absence of genetic variation. Nevertheless, the environmental factors strongly influence on phenotypic effect.

Inflammation primarily results in a reduction in milk production. Seegers et al. [2003] reported that the total loss in milk production arising from clinical mastitis was 375 kg, i.e. approximately 5% of lactation yield. However, production losses were highly variable and mainly eventuate from a lactation period in which cows suffering from mastitis.
Table 2. Heritability estimates ($h^2$) and genetic correlations ($r$) among lactational somatic cell score (SCS) [Dube et al., 2008]

<table>
<thead>
<tr>
<th>Heritability estimates</th>
<th>Genetic correlations</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCS$_1$ 0.07±0.01</td>
<td>SCS$_1$ 0.82±0.09</td>
</tr>
<tr>
<td>SCS$_2$ 0.11±0.01</td>
<td>SCS$_2$ 0.85±0.05</td>
</tr>
<tr>
<td>SCS$_3$ 0.11±0.02</td>
<td>SCS$_3$ 0.96±0.06</td>
</tr>
</tbody>
</table>

SCS$_1$ – SCS for first lactation; SCS$_2$ – SCS for second lactation; SCS$_3$ – SCS for third lactation.

Table 3. Heritability estimates ($h^2$) for somatic cell count and mastitis

<table>
<thead>
<tr>
<th>$h^2$</th>
<th>Parameter</th>
<th>Additional information</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.16</td>
<td>SCC</td>
<td>Holstein</td>
<td>Welper and Freeman, 1992</td>
</tr>
<tr>
<td>0.18</td>
<td>SCC</td>
<td>Danish Holstein</td>
<td>Lund et al., 1994</td>
</tr>
<tr>
<td>0.11±0.04</td>
<td>SCC</td>
<td></td>
<td>Mrode and Swanson, 1996</td>
</tr>
<tr>
<td>0.10-0.14</td>
<td>SCC</td>
<td></td>
<td>Mrode et al., 1998</td>
</tr>
<tr>
<td>0.136±0.028</td>
<td>SCC</td>
<td>during the first lactation for all lactations</td>
<td>Sender, 2001</td>
</tr>
<tr>
<td>0.06±0.02</td>
<td>SCS</td>
<td>Finnish Ayrshire</td>
<td>Ikonen et al., 2004</td>
</tr>
<tr>
<td>0.11-0.22</td>
<td>SCS</td>
<td></td>
<td>Ptak et al., 2007</td>
</tr>
<tr>
<td>0.07</td>
<td>SCC</td>
<td>in primiparous cows</td>
<td>Dube et al., 2008</td>
</tr>
<tr>
<td>0.11</td>
<td>SCC</td>
<td>in multiparous cows</td>
<td></td>
</tr>
<tr>
<td>0.14-0.15</td>
<td>SCC</td>
<td>Danish Holstein, Danish Red and Danish Jersey</td>
<td>Norberg et al., 2009</td>
</tr>
<tr>
<td>0.07-0.14</td>
<td>SCC</td>
<td></td>
<td>Ptak et al., 2009</td>
</tr>
<tr>
<td>0.05 (0.01-0.11)</td>
<td>SCC</td>
<td></td>
<td>Burlina Penasa et al., 2010</td>
</tr>
<tr>
<td>0.15</td>
<td>SCS</td>
<td>lactations 1 and 2</td>
<td>National Research Institute of Animal Production, Poland, 2010</td>
</tr>
<tr>
<td>0.20</td>
<td>SCS</td>
<td>lactation 3</td>
<td></td>
</tr>
<tr>
<td>0.09-0.18</td>
<td>SCC</td>
<td>lactations 1-3</td>
<td>Rzewuska et al., 2011</td>
</tr>
<tr>
<td>0.11-0.14</td>
<td>SCC</td>
<td>daily average for</td>
<td></td>
</tr>
<tr>
<td>0.025</td>
<td>mastitis</td>
<td>lactations 1-3</td>
<td>Lund et al., 1994</td>
</tr>
<tr>
<td>ca. 0.04</td>
<td>mastitis</td>
<td>Holstein-Friesian</td>
<td>Mrode and Swanson, 1996</td>
</tr>
</tbody>
</table>
Table 3. (Continued)

<table>
<thead>
<tr>
<th>h²</th>
<th>Parameter</th>
<th>Additional information</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02-0.04</td>
<td>clinical mastitis</td>
<td></td>
<td>Rupp and Boichard, 1999</td>
</tr>
<tr>
<td>0.01-0.17</td>
<td>mastitis</td>
<td></td>
<td>Pösö and Mäntysaari, 1996; Rupp and Boichard, 1999; Carlen et al., 2004; Bloemhof et al., 2009</td>
</tr>
</tbody>
</table>

SCC – somatic cell count; SCS – somatic cell score (natural logarithm of SCC).

Table 4. Genetic (r_g) and phenotypic (r_p) correlation coefficients between milk yield and SCC

<table>
<thead>
<tr>
<th>Correlation coefficient (r)</th>
<th>Type of correlation</th>
<th>Additional information</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>-0.16 (for SCS)</td>
<td>genetic phenotypic</td>
<td>primiparous cows Holstein</td>
<td>Welper and Freeman, 1992</td>
</tr>
<tr>
<td>-0.02 (for SCS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.14±0.04 (for SCS)</td>
<td>genetic</td>
<td></td>
<td>Mrode and Swanson, 1996</td>
</tr>
<tr>
<td>-0.07 (for SCS)</td>
<td>genetic phenotypic</td>
<td>test-day milk yield Finnish Ayrshire</td>
<td>Ikonen et al., 2004</td>
</tr>
<tr>
<td>-0.13 (for SCS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.19 (for SCC)</td>
<td>beside genetic (phenotypic)</td>
<td></td>
<td>Sawa et al., 2007</td>
</tr>
<tr>
<td>0.12 (-0.48-0.91) (for SCS)</td>
<td>genetic</td>
<td>test-day milk yield Burlina</td>
<td>Penasa et al., 2010</td>
</tr>
<tr>
<td>-0.55 (for SCC)</td>
<td>genetic phenotypic</td>
<td>lactational milk yield Friesian</td>
<td>El-Awady and Oudah, 2011</td>
</tr>
<tr>
<td>-0.47 (for SCC)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-0.157 (for SCC, see Table 5)</td>
<td></td>
<td>total for Polish Holstein-Friesian, Simmental and Jersey</td>
<td>Litwińczuk et al., 2011</td>
</tr>
<tr>
<td>-0.22 – -0.16 (for SCC)</td>
<td></td>
<td></td>
<td>Jakiel et al., 2011</td>
</tr>
</tbody>
</table>

SCC – somatic cell count; SCS – somatic cell score (natural logarithm of SCC).

Thus, Hagnestam et al. [2007] noticed that the reduction in milk production in the period of 305-day lactation ranged from 0 to 902 kg, depending on the stage of lactation and subsequent lactation, in which the
Swedish Holstein and Swedish Red cows become ill. Hortet et al. [1999] analyzed 32,148 control milkings from 4,968 cows of French Holstein breed, in the milk samples in which SCC was lower than 600 thous. cells ml\(^{-1}\). Value of 50 thous. cells ml\(^{-1}\) was accepted as a reference level of somatic cell. The authors found that daily milk yield of primiparous cows decreased by 0.30 kg at the SCC raise up to 100 thous. cells ml\(^{-1}\), by 0.61 kg at SCC to 200 thous. cells ml\(^{-1}\) and by 1.09 kg at SCC to 600 thous. cells ml\(^{-1}\). Numerous studies (Table 4) show that an increase in the somatic cell count affects the losses of daily milk production to a lesser extent (r\(_p\) from -0.02 to -0.19). Nevertheless, in a case of lactational milk production, however, higher negative correlation (r=-0.55) exists.

The study of Litwińczuk et al. [2011] indicate on sundry sensitivity of cow breeds to an increase of SCC in milk, that is confirmed by differences in correlation coefficients obtained between the SCC and daily yield. Out of three breeds, i.e. Polish Holstein-Friesian, Simmental and Jersey, Polish Holstein-Friesian cows were distinguished by the highest sensitivity to udder infections (r=-0.245 at P≤0.01). At the other two cow breeds (characterized by lower productivity), i.e. Simmental and Jersey, the values of r were substantially lower (r=-0.123 and r=-0.148, respectively).

Increase in the somatic cell count in milk also affects the changes in the basic composition. The most sensitive milk component to mammary gland inflammation is lactose. It is responsible, along with some elements (primarily Na and Cl), for regulating the osmotic pressure in udder, which must be in balance with osmotic pressure of blood [Bleck et al., 2009; Litwińczuk, 2012]. Rise in SCC effects on decline in lactose content, as the result of synthetic activity of mammary gland reduced [Ikonen et al., 2004; Bleck et al., 2009]. This leads to flow of higher quantity of Cl\(^{-}\) and Na\(^{+}\) from blood to milk and K\(^{+}\) into the blood to compensate for the osmotic pressure in mammary gland [Bansal et al., 2005; Litwińczuk, 2012]. Forsbäck et al. [2009] reported that at the SCC level ≤100 thous. ml\(^{-1}\) (healthy udder) lactose content was 4.66 g 100 ml\(^{-1}\) and at the SCC>200 thous. ml\(^{-1}\) (threshold physiological limit) – 4.51 g 100 ml\(^{-1}\). The research of Barłowska et al. [2009], carried out on milk obtained from four breeds of cows (Black-White and Red-White variety of Polish Holstein-Friesian, Simmental and Whiteback), proves that a significant decrease in lactose content also followed by the cellular element level ≥200 thous. ml\(^{-1}\). Nevertheless, the highest decline in this component percentage (by 0.24%) was recorded at the level of SCC ≥1 million ml\(^{-1}\) (P≤0.01), while in the Black-White variety of Polish breed Holstein-Friesian cows it was the lowest (by 0.13%) and the highest in Whiteback (0.35%). The similar tendencies were
noticed by Król et al. [2010] and Litwińczuk et al. [2011] in milk of cows of the greatest importance in global milk production (Holstein-Friesian, Simmental and Jersey). Ogola et al. [2007] demonstrated also the reduction in lactose content from 48.8 g l\(^{-1}\) at the SCC level <250 thous. ml\(^{-1}\) to 43.8 g l\(^{-1}\) at SCC>750 thous. ml\(^{-1}\). Relatively high negative correlation coefficients between somatic cell count in milk and lactose concentration (from \(r=0.38\) to \(r=-0.59\)) determined by a number of authors are a confirmation of this relationship [Zumbo et al., 2004; Bansal et al., 2005; Sawa et al., 2007; Bleck et al., 2009; Pazzola et al., 2012]. Juozaitiene et al. [2004] indicated that a correlation coefficient between SCC in milk and lactose concentration was relatively high and oscillated between -0.38 in lactation I and -0.44 in lactation III (\(P<0.01\)), while this dependence for protein or fat content was very low and found within -0.1 – -0.04 range. Lindmark-Månsson et al. [2000] reported (as a few), though, a totally different relationships between log\(_{10}\)SCC and lactose percentage (0.073) and higher for fat, and protein level, respectively: \(r=0.277\) and \(r=0.374\) (in milk of Swedish Holstein cows).

With the increase in somatic cell count in milk protein percentage is generally unchanged or could slightly rise. However, significant changes occur in particular fractions. Accordingly to Lindmark-Månsson et al. [2006], concentration of total protein usually remains unchanged at SCC to 1 million ml\(^{-1}\). Whereas, the results of Litwińczuk et al. [2011], obtained on the basis of research performed on milk of cows of four breeds, indicates that content of this component decreased to SCC level in the range of 401-500 thous. ml\(^{-1}\), and then slightly increased (at SCC: 501-1,000 thous. ml\(^{-1}\)). El-Awady and Oudah [2011], analyzing monthly and lactational losses in protein production in the milk of Friesian cows, stated that they successively followed and occurred at SCC level >100 thous. ml\(^{-1}\). At SCC>2 million ml\(^{-1}\) the losses amounted to 2.12 kg monthly and 21.0 kg during lactation. The results of other authors indicate that there is no clear correlation between somatic cell count and protein content [Ikonen et al., 2004; Ogola et al., 2007; Barłowska et al., 2009; Forsbäck et al., 2009]. Generally low correlation coefficients obtained are (as already was mentioned) the confirmation of absence of an unequivocal relationship between SCC and protein content.

There is also no unequivocal opinion among researchers in the case of the relationship between somatic cell count and fat content in milk. Król et al. [2010] pointed at a downward tendency of this component with an increase in SCC, but the differences were generally statistically insignificant in almost all breeds. However, Barłowska et al. [2009] indicated a reverse tendency. In the opinion of Ogola et al. [2007] and Forsbäck et al. [2009], changes in protein...
and fat content in milk at elevated somatic cell count aroused from an increased risk of proteolysis and lipolysis. El-Awady and Oudah [2011], analyzing monthly and lactational wastage in fat production in the milk of Friesian cows, stated that the losses occurred at SCC level >100 thous. ml⁻¹. At SCC>2 million ml⁻¹ the losses amounted to 3.01 kg monthly and 27.9 kg during lactation. According to sundry authors [Lindmark-Månsson et al., 2000; Ikonen et al., 2004; Zumbo et al., 2004; Sawa et al., 2007; Pazzola et al., 2010], the correlation coefficients between SCC and fat content in milk are generally low and oscillated from negative (r=−0.05) to positive (in a range of r=0.07 to r=0.37). Whereas in the research of Sender et al. [2001], the genetic correlations between somatic cell count and yield of fat and protein were positive and higher in the first lactation (r=0.39) than those estimated for all lactations (r=0.16).

Heuer et al. [1999] and Windig et al. [2005] postulated that a higher risk of mastitis incidence appeared with an elevation in fat to protein ratio in milk. According to Heuer et al. [1999], the acute inflammatory response is more likely to occur when 1.5 value of this ratio has been exceeded.

5. SCC AND MILK WHEY PROTEINS

Somatic cell count also affects the changes in whey protein content. They represent 20-25% of milk proteins, including approximately 75% of albumin, i.e. α-lactalbumin (α-LA), β-lactoglobulin (β-LG) and bovine serum albumin (BSA). The remainder constitutes immunoglobulins, protezo-peptones, glycomacropeptides, lactoferrin, growth factors, hormones and various enzymes – including lysozyme. These compounds are globular proteins with a three-dimensional structure. It should be noted that the aforementioned proteins, except BSA and immunoglobulins deriving from the blood, are synthesized in mammary gland of cow [Farrell et al., 2004; Chatterton et al., 2006; Michaelidou and Steijns, 2006; El-Loly and Farrag, 2007; Smithers, 2008].

Whey proteins demonstrate multidirectional pro-health effects on the human body. They affect, among others, the digestive, immune, cardiovascular and nervous systems and play a significant role in reducing a risk of occurrence of various social diseases [Pan et al., 2006; Liu et al., 2007; Król et al., 2008; Smithers, 2008].

The dominant whey protein presenting in the milk of cows and other ruminants is β-lactoglobulin. It plays an important antioxidant role in milk. It
also exhibits anticancerogenic, antiviral and antibacterial properties. With the immunoglobulins G β-LG is probably involved in forming the passive immunity [Sutton and Alston-Mills, 2006; Hernández-Ledesma et al., 2008]. α-lactalbumin actively contributes in a control of lactation and milk secretion, forming together with galactosyltransferase (GT) an indispensable component of lactose synthetase. It also shows anticancerogenic, antibacterial (against Gram-positive) and antiviral activity. Furthermore, α-La functions as an immunological factor, inter alia, modulating (increasing) a neonatal immunity [Séverin and Wenshui, 2005; Zimecki and Artym, 2005; Chatterton et al., 2006; Riley et al., 2008; Kanwar et al., 2009]. Thus, bovine serum albumin in milk is physically and immunologically identical to that of blood serum albumin, as it is not synthesized in the mammary secretory cells but derives from the so-called nonspecific “leakage” from the blood to milk. According to Litwińczuk et al. [2011], BSA concentration in milk is an indicator of permeability of the blood-milk barrier in the mammary gland. Mammary gland inflammations are a major factor increasing the permeability of cell membranes of the udder. Furthermore, lactoferrin is also involved in a natural defense of body against bacterial infections. By binding and sequestering of iron, lactoferrin exhibits antibacterial properties against Gram-positive and Gram-negative bacteria, non-capsular and capsular viruses as well as various types of fungi and parasites. It functions both bacteriostatic and bactericidal against Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Clebsiella pneumonia. An advantage of lactoferrin in a fight against bacterial infections is possibility of increasing a bacteria sensitivity to certain antibiotics (vancomycin, penicillin) and lowering their effective doses. The combination of penicillin with lactoferrin doubles the inhibitory activity of antibiotic against Staphylococcus aureus [Diarra et al., 2002]. Lactoferrin also determines the innate immune.

The increase in concentration of this protein (cow milk contains an average of 20-200 mg dm⁻³) is not only due to the secretion of colostrum and development of the mammary glands but also it testifies of occurring infections, inflammations or injuries. Lactoferrin is a component of the secondary granules of neutrophils, where in the case of an injury, infection or inflammation is released into the blood [Baker and Barker, 2005; Artym, 2010; Garcia-Mantoya, 2011]. Large variability of single-nucleotide polymorphism – SNPs (more than 140) in the lactoferrin gene indicates that there is a high probability of existing of a mastitis-resistance marker or eventually also a marker of milk yield in this gene [Wojdak-Maksymiec et al., 2006; Żukiewicz et al., 2012]. Furthermore, this protein determines the
maturation of immune cells. Besides lactoferrin, lysozyme also occurs to be one of the most crucial component of the non-specific humoral immune response, i.e. as a bactericidal component of various glandular secretions, including mammary gland. Concentration of the protein varies according to an udder health, i.e. is much higher in cow colostrum and mastitis milk than in normal milk, what can be used in the mastitis diagnosis. Synergistic effect of the enzyme with immunoglobulins and lactoferrin against *Escherichia coli* and *Micrococcus luteus* was also noticed [Séverin and Wenshui, 2005; Moatsou, 2010]. Immunoglobulins are very important group of proteins exhibiting antimicrobial activity. These compounds are globulins of high molecular weight and occur in plasma and body fluids. Three major classes of immunoglobulins are distinguished, i.e. IgG, IgM and IgA, depending on physical-chemical structure and biological activity. IgG dominate in milk of ruminants (approximately 80%), while IgA in other mammalian milk, including human (approximately 90%). They determine the specific humoral immunity of the body [El-Loly and Farrag, 2007]. During the process of antigens binding as well as phagocytosis or complement activation these proteins are involved in the destruction of pathogenic microorganisms, i.e. *Escherichia coli*, *Candida albicans*, *Clostridium difficile*, *Shigella flexneri*, *Streptococcus mutans* and *Helicobacter pylori*. Furthermore, immunoglobulins block the action of toxins and viruses [Gapper et al., 2007].

The study of Litwińczuk et al. [2011], carried out on milk of cows of four breeds, i.e. Black-White and Red-White variety of Polish Holstein-Friesian, Simmental and Jersey, proved an effect of SCC on changes in whey protein content (Table 6). It was found that the rise of somatic cell count decreased the content of major albumins, i.e. α-LA and β-LG, to a small extent and that was confirmed by very low, statistically insignificant correlation coefficient obtained (r=-0.07 and r=-0.05, respectively). Negative value of the correlations indicates, however, the direction of changes and may suggest that in more advanced disease states a decline in these protein concentrations in milk can be higher. Wickström et al. [2009], found a significant decrease in α-LA concentration with an increase in somatic cell count in bulk tank milk obtained in Sweden.

The authors also observed a reduction in β-LG concentration, along with the decline in α-LA content. Similar tendencies were obtained in the study of Bleck et al. [2009], including two herds maintained in U.S., i.e. the University Illinois herd and the University Minnesota herd, wherein for the first herd achieved a negative correlation between logSCC and α-LA (r=-0.059) and for the second herd – positive (r=0.177).
Table 5. Correlation coefficients (r) between SCC and milk yield as well chosen milk components [Litwińczuk et al., 2011; Król et al., 2012]

<table>
<thead>
<tr>
<th>Breed</th>
<th>Milk yield</th>
<th>Casein</th>
<th>BSA</th>
<th>Lactoferrin</th>
<th>Lysozyme</th>
<th>IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polish Holstein-Friesian</td>
<td>-0.245***</td>
<td>-0.573***</td>
<td>0.711***</td>
<td>0.687***</td>
<td>0.694***</td>
<td>-</td>
</tr>
<tr>
<td>Simmental</td>
<td>-0.123</td>
<td>-0.691***</td>
<td>0.577***</td>
<td>0.710***</td>
<td>0.603***</td>
<td>-</td>
</tr>
<tr>
<td>Jersey</td>
<td>-0.148</td>
<td>-0.723**</td>
<td>0.472</td>
<td>0.540**</td>
<td>0.693**</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>-0.157</td>
<td>-0.591***</td>
<td>0.591***</td>
<td>0.652***</td>
<td>0.632***</td>
<td>0.790</td>
</tr>
</tbody>
</table>

* – significant at P<0.01; *** – significant at P<0.001.

Table 6. Comparison of values (%) of normal milk with that of mastitis milk having high somatic cell count [Jones and Bailey, 2009; Litwińczuk et al., 2011]

<table>
<thead>
<tr>
<th></th>
<th>Jones and Bailey, 2009</th>
<th>Litwińczuk et al., 2011</th>
<th>Direction of changes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal milk</td>
<td>Milk with high SCC</td>
<td>Normal milk</td>
</tr>
<tr>
<td>Fat</td>
<td>3.5</td>
<td>3.2</td>
<td>-</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.9</td>
<td>4.4</td>
<td>-</td>
</tr>
<tr>
<td>Total protein</td>
<td>3.61</td>
<td>3.56</td>
<td>3.75-4.08</td>
</tr>
<tr>
<td>Casein</td>
<td>2.80</td>
<td>2.30</td>
<td>2.81-3.14</td>
</tr>
<tr>
<td>Whey proteins</td>
<td>0.8</td>
<td>1.3</td>
<td>-</td>
</tr>
<tr>
<td>Serum albumin</td>
<td>0.02</td>
<td>0.07</td>
<td>0.04-0.05</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>0.020</td>
<td>0.100</td>
<td>0.007-0.009</td>
</tr>
<tr>
<td>Immunoglobulins</td>
<td>0.1</td>
<td>0.6</td>
<td>-</td>
</tr>
<tr>
<td>α-LA</td>
<td>-</td>
<td>-</td>
<td>0.100-0.120</td>
</tr>
<tr>
<td>β-LG</td>
<td>-</td>
<td>-</td>
<td>0.31-0.38</td>
</tr>
</tbody>
</table>

Content of immunoactive proteins (lactoferrin and lysozyme) and bovine albumin serum increases, however, with a rise of somatic cell count. In the research of Litwińczuk et al. [2011], milk with the highest SCC (501-1,000 thous. ml\(^{-1}\)) included averagely more lactoferrin (by 34.8%), lysozyme (by 60.2%) and BSA (by 64.7%), in comparison to milk with SCC below 100 thous. ml\(^{-1}\). The authors confirmed a substantial effect of SCC on immunoactive proteins and bovine albumin serum content by relatively high
positive values of the correlation coefficients calculated. For lactoferrin content, \( r \) was amounted to 0.65, for lysozyme – \( r=0.63 \) and BSA – \( r=0.59 \). In the case of bovine albumin serum, these correlations were clearly differentiated at certain breeds of cows (Table 5). The highest relationship, i.e. at the level of \( r=0.711 \), was obtained for Holstein-Friesian cows, while a noticeably lower dependence was found for Simmental (\( r=0.577 \)) and Jersey cows (\( r=0.472 \)). Higher sensitivity of Holstein-Friesian cows to udder infections was manifested by significantly greater drops in their daily yield of milk. Therefore, it can be assumed that in Simmental and Jersey cows the SCC growth is not followed by such intensive permeation of bovine serum albumin from blood to milk, as it was observed in Holstein-Friesian cows. This would indicate a higher resistance of Simmental and Jersey cows to mammary gland infections. Furthermore, Urech et al. [1999], in the study of quarter milk, noticed similar tendencies when 100 thous. cells ml\(^{-1}\) were recognized as the threshold limit of somatic cell count. Quarter milk, obtained from clinically healthy mammary glands, contained an average of 84 thous. somatic cells ml\(^{-1}\), whereas the milk from infected glands included 293 thous. cells ml\(^{-1}\). The authors showed the significant rise in the content of lactoferrin (by 0.45%), BSA (by 0.10%) and immunoglobulins (by 0.39%) in milk from affected udder. Hamann [2002] defined “the gold standard” for SCC at the level of 100 thous. ml\(^{-1}\). Higher somatic cell count testifies, in his opinion, to a dysfunctional milk secretion, what leads to reduction in daily milk production, changes in milk chemical composition and deterioration of technological properties. However, similar tendencies and statements are also included in the papers of other authors [Piccinini et al., 2006; Berlung et al., 2007; Barłowska et al., 2009; Forsbäck et al., 2009]. Lindmark-Månsson et al. [2000 and 2006] observed that a somatic cell count of over 5 thous. ml\(^{-1}\) increases lactoferrin content in milk, and a close relationship between this component and udder health status has been confirmed by very high correlation coefficients between milk lactoferrin concentration and somatic cell count (\( r=0.962 \) and \( r=0.918 \)) obtained in two independent studies. Furthermore, Nudda et al. [2001], in the research carried out on the milk obtained from Sarda sheep, also showed the significant differences in whey protein content between SCC groups, and for lactoferrin, BSA and IgG the significant positive correlations with somatic cell count were noticed, amounting respectively: \( r=0.39 \), \( r=0.31 \) and \( r=0.35 \). Higher correlation coefficient between SCC and BSA (\( r=0.53 \)) was stated in the research of Poutrel et al. [1983], conducted on milk of Holstein and Friesian-Holstein breed. Król et al. [2012] received, however, high correlation coefficient between SCC and IgG (\( r=0.790 \)). A significant increase in
lactoferrin, albumin and IgG content with deterioration of mammary gland health status was also confirmed in the research of other authors [Leitner et al., 2004; Piccinini et al., 2006; Liu et al., 2007].

6. SCC AND TECHNOLOGICAL PARAMETERS OF MILK

Raw material intended for processing should be characterized by the appropriate technological indicators, predisposing it to produce a dairy product. It should be noticed that basic chemical composition, acidity, thermal stability of milk and rennet clotting time are the most major indicators.

In the production of many dairy products, content of non-fat dry matter, especially total protein, including casein, is considered to be of great importance. Casein fraction determines the clotting time, curd firmness and cheese yield from a milk volume specified. \( \kappa \)-casein is the sole casein fraction sensitive to an addition of rennet (chymosin enzyme). However, it has no sensitivity to calcium presence. \( \kappa \)-casein stabilizes other casein fraction toward calcium, forming with them the micelles. It is precipitated at pH value 4.6 at 20°C. \( \kappa \)-casein specific feature is the ability to coagulate, both enzymatic and acid, which is used in the production of rennet and curd cheese. Raw material with high protein content is also needed in the production of fermented beverages as it is responsible for binding an appropriate amount of water in product. As a consequence, the product is stable and resistant to syneresis. The whey protein with an importance in milk processing is \( \beta \)-lactoglobulin. During the thermal treatment it is denatured, causing an exposure of -SH groups. -SH groups bind the metal ions (especially copper and iron) and as a result they inhibit the oxidation of milk fat in dairy products. The negative effect of \( \beta \)-lactoglobulin denaturation is a binding of the molecules denatured with \( \kappa \)-casein. Nevertheless, the binding hinders the process of enzymatic coagulation. In the cheese production also a great importance has protein to fat ratio. Furthermore, lactose presence in milk enables an acidification of milk, owing to the fact that as a sugar lactose is a food for lactic acid bacteria [Barłowska, 2007].

The preparation of stable dairy products such as UHT milk and cream, sterilized drinking milk, unsweetened condensed milk and fermented beverages requires a high temperature or a little lower but with a longer time of duration. With regard to this, the raw material which is capable of physical endurance of heat treatment used is needed, and thus of a high thermal stability. This term is generally understood as the ability to retain the colloidal
properties of milk, in particular proteins, during high temperature operation. In fact, low stability may cause sedimentation of proteins denatured in the final product and occasionally even its gelification during the storage [Singh and Creamer, 1992; Kruk, 2001; Faka et al., 2009]. Thermal stability of milk is not constant and depends directly on its chemical composition and physical characteristics. Milk as a raw material meets the quality criteria in this regard if it withstands the time of heat treatment in temperature of 140°C≥8 min [Kruk, 2001; Litwińczuk, 2012]. One of the direct factors that determine thermal stability of milk is its acidity. The research of Barłowska et al. [2010] indicates a significant negative correlation between active acidity (pH values) and thermal stability of milk (r=-0.30). Thermal stability of milk increases in the pH value range from 6.4 to 6.7 and at pH value above 6.7 it begins to reduce significantly, reaching a minimum at pH value 6.9. The parameter value begins to rise afresh at pH>6.9 [Singh and Fox, 1986, 1987; Rattay and Jelena, 1996]. Singh and Fox [1985a, b, 1986, 1987] explained the reduction in thermal stability of milk at pH value proximate to 6.9 as the result of dissociation of κ-casein complex with whey proteins. This contributes to higher sensitivity of micelles to high temperature and calcium ions operation [Singh, 1995]. van Boekel et al. [1989a, b] stated that high thermal stability of casein micelles at pH<6.7 (where whey proteins deposit and bind) was caused by binding of calcium ions in the initial phase of the process, which reduced the concentration of Ca²⁺ in milk. Furthermore, thermal stability of milk is determined by the content of whey proteins and calcium ions. In milk with normal concentration of whey proteins a protective activity of these proteins in relation to casein and vice versa, i.e. casein with regard to whey proteins, is observed.

On the one hand, whey proteins denatured undergo a microfloculation on the casein micelle surface (in the order: serum albumin, β-lactoglobulin and α-lactalbumin, according to the heat resistance), which prevents their stronger aggregation and loss out of solution. On the other hand, they block calcium access to the micelles (by interacting with casein) and as a result milk obtains better stability. The micelle size also greatly influences on milk heat stability. Small micelles contain relatively more κ-casein which stabilizes the remaining casein fractions to calcium ions. Therefore they are more resistant to high temperature (140°C) [O’Connel and Fox, 2000]. Barłowska et al. [2010] reported significant positive correlation between milk thermal stability and rennet clotting time (r=0.32).

A crucial indicator determining the suitability of milk for rennet cheese production is rennet clotting time. Defective milk, i.e. obtained from cows
with udder inflammation, may clot after 20 minutes or does not coagulate at all. The rate of clot formation and degree of firmness are largely dependent on the casein content in milk [Ikonen et al., 2004], proportion of calcium to nitrate compounds (0.2 – for slowly coagulating milk and 0.23 – for normal and quickly coagulating milk) and casein micelle size [Litwińczuk, 2012]. It is considered that rennet clotting time of milk is dependent on protein content, in that casein primarily. Nevertheless, research results are quite divergent with regard to these relationships. Analyzing milk of Finnish Ayrshire cows, Ikonen et al. [2004] stated the relationship between coagulation time (or curd firming time) and content of protein and casein. Similar analyses, which were performed by Olofsson et al. [1992] on milk of Friesian cows and Ikonen et al. [1999] on milk of Finnish cows, showed that shorter coagulation time was associated with lower protein content. However, Olofsson et al. [1992], evaluating milk of Angler cows, did not show any dependences. Results of curd firmness assessment obtained by the various authors were also divergent. In Olofsson et al. [1992], high values for curd firmness correlated with high protein and casein content, whereas Ikonen et al. [1999, 2004] indicated a reverse tendency, i.e. high values for curd firmness correlated with low protein and casein content. Ability of milk to enzymatic coagulation depends greatly on the size of casein micelles. Large micelles contain relatively less \( \kappa \)-casein, which functions as a stabilizer for micelle particles, and therefore milk coagulates quickly [Ziajka, 2008]. Content of calcium in milk is considered of great importance to enzymatic coagulation processes [Nájera et al., 2003; Barłowska et al., 2010]. Milk coagulation time significantly affects the firmness of curd formed. Ikonen et al. [2004] confirmed this strong relationship by very high correlation coefficients \( r_g=0.97 \) and \( r_p=0.92 \).

Breed of cows is crucial factor determining milk suitability to cheese production [Okigbo et al., 1985a, b; Malossini et al., 1996; Tyrisevä et al., 2004]. Research of numerous authors indicate that cows of local breeds produce milk which faster coagulates and curd obtained is more firm, in comparison with highly productive breeds [Chiofalo et al., 2000; Barłowska and Litwińczuk, 2006; De Marchi et al., 2007; Litwińczuk et al., 2012]. Furthermore, in the latter a large proportion of noncoagulating milk samples is stated. Ikonen et al. [2004] found that in 4,700 milk samples collected from Finnish Ayrshire cows approximately 13% of them (618 samples) had not coagulated, i.e. they had not aggregated and formed any curd. Kübarsepp et al. [2003] stated 8% of noncoagulating milk samples in dairy cattle breeds raised in Estonia (Estonian Holstein, Estonian Red and Estonian native breeds) and
Somatic Cell Count as the Factor Conditioning Productivity …

slightly more (11%) in Red and White Holsteins. Ikonen [2000], however, did not notice such problem in milk of Holstein-Friesian and Finnish cows.

Casein, as an essential milk component deciding about its suitability for processing, particularly for cheese production, usually declines in inflammations with accordance to mastitis status. This is due to the fact that protein fraction is synthesized whole in the mammary gland. Mammary gland infection results in the changes in milk component secretion, inter alia, the noncasein fraction (NCN fraction) is found to be elevated, while the casein content is decreased. It occurs partly due to increased proteolysis leading to a reduction in the casein to total protein ratio in infected quarters. And this may be linked to increased endogenous proteolysis which eventuates from the elevation of plasmin or other proteases derived from somatic cells.

The result of increased permeability of mammary epithelium is the influx of blood proteins (immunoglobulins, especially IgG, and bovine serum albumin) into milk, which results in an elevated NCN content [Ogola et al., 2007]. Forsbäck et al. [2009], analyzing milk of Swedish Red Breed and Swedish Holstein, confirmed the fact of reduction in casein content in milk with somatic cell count rise, i.e. from 2.61% at SCC<100 thous. ml\(^{-1}\) to 2.56% at SCC>300 thous. ml\(^{-1}\) (P≤0.05). In the research of Litwińczuk et al. [2011], carried out on milk of cows of four breeds, i.e. Black-White and Red-White variety of Polish Holstein-Friesian, Simmental and Jersey, a downward tendency for casein content was also showed. Its level declined in milk with SCC rise, regardless of the breed of cows and production season. A distinct negative relationship between milk casein and SCC was confirmed by a relatively high value of correlation coefficient (r=-0.591 at P<0.001). Nevertheless, it should be emphasized that the values of this coefficient were various for particular breeds (Polish Holstein-Friesian – r=-0.573, Simmental – r=-0.691 and Jersey – r=-0.723), which indicates the sundry sensitivity of these cow breeds to udder inflammations.

As already mentioned, the milk acidity has significant effect on its resistance during heat treatment. Normal (fresh) milk, derived from cows with healthy udder, should have pH range from 6.5 to 6.8. Such pH guarantees the colloidal stability of milk [Litwińczuk, 2012]. Many authors [Ikonen et al., 2004; Bansal et al., 2005; Ogola et al., 2007] indicated the rise in values of active acidity in milk taken from infected quarters. Higher levels of citrate and bicarbonate found during udder inflammation may be responsible for elevated pH levels. Ogola et al. [2007] showed the increase in pH value to 6.81 in milk with elevated somatic cells count (SCC>750 thous. ml\(^{-1}\)), while in milk contained less than 250 thous. ml\(^{-1}\) this value was equal to 6.63. However, the
differences were statistically insignificant. Results obtained by Ikonen et al. [2004] confirmed these relationships by the positive correlation coefficients between SCC and pH values ($r_g=0.16$ and $r_p=0.26$).

Udder inflammations also cause the changes in proportions of milk proteins and mineral components, and consequently the reduction of milk colloidal stability. In mastitis milk content of whey proteins increases, and as the result a protective effect of casein in relation to them becomes insufficient. This causes an imbalance between $\kappa$-casein and $\beta$-lactoglobulin, for which the optimum molar ratio should be equal to 1 in normal milk [Żbikowska and Szerszunowicz, 2002]. Furthermore, an increase in Ca and P content in milk also lowers the thermal stability.

The balance between calcium and magnesium ions as well as phosphates and citrates is greatly important for this parameter of milk technological suitability [Ziajka, 2008].

Increase of SCC in milk causes an elongation of rennet clotting time or its absence. Longer coagulation time of milk results in a decrease of curd firmness, impairment of syneresis processes, raised cheese moisture and reduced cheese yield efficiency (high losses of casein which passes to whey). This is mainly due to the changes in content of milk components (the changes in distribution of proteins, including casein, decline in lactose content, changes in concentration of minerals, mainly calcium reduction).

The fact of clotting time lengthening due to milk SCC increase confirms the results of many authors [Nudda et al., 2001; Ikonen et al., 2004; Zumbo et al., 2004; Barłowska et al., 2009]. Ikonen et al. [1999, 2004] indicated strong genetic correlations between milk coagulating properties (MCP), i.e. rennet clotting time (RCT), curd firming time ($k_{20}$) and curd firmness ($a_{30}$), and SCS estimated for cows of Finnish Ayrshire breed. They suggested that selection for low SCC could improve MCP and reduce the occurrence of noncoagulating milk samples. On the basis of the research of Barłowska et al. [2009], conducted on cow milk of four breeds (Black-White and Red-White variety of Polish Holstein-Friesian, Simmental and Whiteback), it should be also emphasized that cows of Simmental and native Polish Whiteback breed produced milk of noticeably better suitability to cheese production. It was proved by evidently shorter milk enzymatic coagulation time (moment of the first flakes of casein loss) for Simmental and Whiteback breed, which amounted from 4:32 to 5:41 min at SCC<400 thous. ml$^{-1}$, in comparison with milk of Holstein-Friesian cows (from 7:05 to 8:32 min) – Table 7.
They also showed that milk with somatic cell count above 1,000 thous. ml⁻¹ had longer coagulation time by approximately 20-30% (depending on breed), with regard to the milk samples at SCC from 201 to 400 thous. ml⁻¹. Ikonen et al. [2004] confirmed these strong relationships between SCC and milk technological parameters to cheese production by genetic correlation coefficients obtained, i.e. between SCC and RCT – \( r_g = 0.29 \) as well as SCC and \( a_{30} - r_g = -0.45 \). With regard to milk of Sarda sheep breed the similar relationships between SCC and coagulation properties of milk were also noted [Nudda et al., 2001]. Rennet clotting time significantly (P<0.01) lengthened from 16.05 min at SCC<300 thous. ml⁻¹ to 23.64 min at SCC>2,000 thous. ml⁻¹. Furthermore, correlation coefficients obtained between SCC and RCT (\( r = 0.43 \)), SCC and \( k_{20} \) (\( r = 0.41 \)) as well SCC and \( a_{30} \) (\( r = -0.43 \)) were statistically significant (P<0.01).

As mentioned above, a right behavior of milk during various technological processes is determined by the content and proportions of individual minerals. Udder inflammations cause a decline in Ca, P and K concentration but level of Cl and Na significantly increases [Bruckmaier et al., 2004; Ogola et al., 2007; Litwińczuk, 2012]. Ogola et al. [2007] analyzed effect of SCC on Ca, K, Na and Cl content in 396 samples of quarter milk taken from cows of Holstein-Friesian and Zebu crossbreed. They showed that Na content was 46.5 mg 100 g⁻¹, K – 146.3 mg 100 g⁻¹, Ca – 119.5 mg 100 g⁻¹ and Cl – 100.5 mg 100 g⁻¹ at SCC<250 thous. ml⁻¹. Rise in SCC over 750 thous. ml⁻¹ elevated Na concentration to 78.5 mg 100 g⁻¹ (by over 68%) and Cl to 183.5 mg 100 g⁻¹ (by over 72%), however, reduced K content to 108.9 mg 100 g⁻¹ (by over 34%) and Ca to 97.8 mg 100 g⁻¹ (by over 22%). Rise in chlorine concentration in mastitis milk follows due to decline in lactose content which is an essential component conditioning milk fermentation and acidification, and as a result also the syneresis processes in cheese grains. This affects an increase in cheese moisture and decrease in whey yield. A major mineral component of milk, which appropriate concentration determines the rennet curd formation, is Ca. It shields negative functional groups on the micelle surface and plays a crucial role in formation of intermicelle calcium bridges [Ziajka, 2008]. Therefore, the decrease in calcium content in mastitis milk is one the factors lengthening its coagulation time or even contributing to the lack of coagulation. As most milk Ca is associated with casein micelles, Ogola et al. [2007] reported that reduced casein content could explain the lowered calcium levels in infected quarters. Increasing of cellular component content in milk negatively affects not only the raw material but also its products. During the ripening process of cheese made from milk with high SCC undesired proteolytic processes occur, which leads to the reduction in quality of cheeses or disqualification [Marino et al., 2005; Wickström et al., 2009].
Table 7. Productivity, basic chemical composition and technological suitability of milk obtained from different cow breeds, with regard to somatic cell count [Barłowska et al., 2009]

<table>
<thead>
<tr>
<th>Breed</th>
<th>SCC group (thous. ml⁻¹)</th>
<th>Specification</th>
<th>Daily milk yield (kg)</th>
<th>Dry matter (%)</th>
<th>Protein (%)</th>
<th>Fat (%)</th>
<th>Protein/Fat</th>
<th>Lactose (%)</th>
<th>Clotting time (min)</th>
<th>Thermal stability (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black-White variety of Polish Holstein-Frisian</td>
<td>I</td>
<td>26.1⁰</td>
<td>13.24⁰</td>
<td>3.40⁰</td>
<td>4.27⁰</td>
<td>0.81</td>
<td>4.91⁰</td>
<td>7:05⁰</td>
<td>4:40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>II</td>
<td>26.7⁰</td>
<td>13.17⁰</td>
<td>3.42⁰</td>
<td>4.26⁰</td>
<td>0.82</td>
<td>4.84⁰</td>
<td>7:52⁰</td>
<td>4:20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>III</td>
<td>23.8⁰</td>
<td>13.62⁰</td>
<td>3.66⁰</td>
<td>4.46⁰</td>
<td>0.84</td>
<td>4.84⁰</td>
<td>7:46⁰</td>
<td>4:51</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>23.7⁰</td>
<td>13.38⁰</td>
<td>3.59⁰</td>
<td>4.36⁰</td>
<td>0.84</td>
<td>4.78⁰</td>
<td>9:46⁰</td>
<td>4:25</td>
<td></td>
</tr>
<tr>
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<td>13.08</td>
<td>3.54³⁻</td>
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<td>3.52³⁻</td>
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<td>0.82³⁻</td>
<td>4.66³⁻</td>
<td>9:20³⁻</td>
<td>4:32</td>
<td></td>
</tr>
</tbody>
</table>

I – to 200 thous. ml⁻¹, II – 201-400 thous. ml⁻¹, III – 401-1000 thous. ml⁻¹, IV – above 1000 thous. ml⁻¹;
A, B, C, a, b, c – differences between SCC within a breed; a, b, c – differences significant at P≤0.05; A,B, C – differences significant at P≤0.01.
CONCLUSION

Udder inflammation remains the most frequent and expensive disease affecting dairy cattle. For the dairy industry the most pernicious are subclinical (non-symptomatic) inflammations, which are very often undiagnosed. As a result, milk with lowered quality becomes a commercial milk. SCC in cow milk is a good indicator of the mammary gland health status, that is why clinical and subclinical inflammations occurred are associated with a significant increase in their number in milk. Inflammations primarily affect a reduction in the milk production. On the basis of the fact mentioned above, it could be concluded that highly productive cow breeds are more susceptible to inflammation, which in turn results in higher losses in milk production in such herds. Decreased synthetic activity of infected mammary gland are also revealed in the changes in individual milk component content and in the ratios between them. As a consequence, milk nutritional value and suitability for processing is reduced.

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Chapter 4

STRATEGIES TO IMPROVE THE REPRODUCTIVE EFFICIENCY OF DAIRY CATTLE

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ABSTRACT

The reproductive performance of a lactating herd is a major component of the profitability of a dairy farm. Factors such as negative energy balance, heat stress and failures in heat detection can severely compromise reproductive parameters. To overcome these problems, a variety of strategies can be used. For example, failures in estrus detection can be solved with the use of fixed-time artificial insemination (FTAI). Progesterone implants combined with estradiol administration are very effective in promoting the onset of a new follicular wave. With the use of a luteolytic agent and an inducer of ovulation, AI can be performed at the appropriate time without the need for estrus observation. In countries where the use of such drugs is not allowed, protocols based on GnRH and PGF2α may also offer optimal synchronization of ovulation. In locations where pregnancy rates are compromised by high temperatures, a viable

∗ Corresponding author: Marcelo Marcondes Seneda, Laboratório de Reprodução Animal, DCV, CCA, Rodovia Celso Garcia Cid, Pr 445, Km 380, State University of Londrina (UEL), Londrina, PR, Brazil, 86051-990, Phone: +55 43 3371-4064, Fax: +55 43 3371-4063, Email: mseneda@uel.br.
alternative to FTAI may be the fixed-time embryo transfer (FTET). Embryos at the morula and blastocyst stage are more resistant to heat stress than gametes and embryos in early stages of development. Thus, embryo transfer (ET) on day 7 of development can ensure satisfactory pregnancy rates throughout the year, even in months and/or regions with higher average temperatures. ET has also been effective in preventing early embryonic mortality and increasing pregnancy rates in repeat breeders. Another strategy that can enhance the reproductive efficiency of dairy herds is the cryopreservation of embryos; embryos are stored and can be used at strategic times, such as in the warmer months of the year. This chapter will discuss technological strategies that can lead to higher breeding efficiency, improved reproductive efficiency and increased profitability of livestock on dairy farms.

1. INTRODUCTION

Over the last 50 years, reproductive efficiency has declined greatly in the dairy industry. Currently, parameters such as conception at first service, services per conception and calving interval have shown to be deteriorating. The worsening of these features can be explained by modifications in the management of dairy farms and by negative genetic correlations between milk production and fertility. This decline in reproductive performance is of great concern to milk producers, given the significance of good fertility to the dairy industry. In addition to adequate nutritional management, which is of major importance to achieving positive results, a variety of strategies can be used to ensure better reproductive outcomes. Fortunately, there is currently enough technology to at least partially reverse this setback. One of the most important approaches developed to improve reproductive efficiency is artificial insemination (AI). In the present context, AI is a hugely widespread technology, and most milk producers use it at some level in their herds. However, the failure to cycle and display estrus frequently leads to unsatisfactory outcomes when only AI is used. Therefore, hormonal protocols have been used to induce estrus and synchronize the moment of ovulation, increasing the percentage of pregnant animals in the herd.

In addition to fixed-time artificial insemination (FTAI), embryo transfer (ET) has provided fairly positive pregnancy rates. This strategy is especially useful in regions with high temperatures because not even FTAI protocols can offer acceptable rates in cattle with heat stress. The transfer of both in vivo and in vitro-produced embryos has been widely used to ensure satisfactory
pregnancy rates throughout the year, even in countries with high temperatures in the summer. This chapter aims to present some of the most promising technologies currently available to improve reproductive efficiency in dairy cattle, as well as their pros and cons. Recent data will be reviewed, and the most suitable situations to use each technique will be discussed.

2. FIXED-TIME ARTIFICIAL INSEMINATION

The detection of estrus is a determining factor for obtaining good pregnancy rates. In dairy herds, over 50% of the cows in heat are not detected due to a lack of mating behavior displayed by the animals (Van Eerdenburg, 2002). High milk production seems to seriously impact this reproductive factor, as low estrus detection rates are even more pronounced among high-producing animals (Jeong et al., 2012). The failure to detect estrus also represents a significant challenge in programs that use cattle with some proportion of Bos indicus blood, as these animals experience an estrus of shorter duration and their estrus frequently begins and ends at night (Bó et al., 2003). In recent years, the use of pharmacological methods to control the estrus cycle (i.e., FTAI protocols) has done away with the need to monitor for estrus. These protocols control the rise of follicular waves and regulate luteal dynamics, promoting synchronization of the moment of ovulation (Nasser et al., 2004). In addition to increasing the number of animals subjected to insemination, FTAI also has brought dairymen great advantages in strategic management of the reproduction of the herd. A wide range of synchronization protocols for FTAI based on a variety of natural and synthetic drugs are available on the market. Some of the most commonly used hormones are prostaglandins, progestagens, estradiol, equine chorionic gonadotropin (eCG), human chorionic gonadotropin (hCG) and gonadotropin-releasing hormone (GnRH).

2.1. Drugs Most Frequently Used for Estrus Synchronization

2.1.1. Prostaglandin

Prostaglandin F\textsubscript{2\alpha} (PGF\textsubscript{2\alpha}) and its synthetic analogues interrupt luteal activity and are widely used to regulate the estrus cycle of lactating dairy cows. The action of this drug depends on how developed the dominant follicle (DF) is at the time of the administration. If the DF is too mature to ovulate,
only the DF of the next wave will be affected, resulting in a longer time span until the next rut occurs.

However, if a DF able to ovulate is present, the manifestation of estrus occurs approximately 48 h after the treatment with PGF$_{2\alpha}$. In general, approximately 50-60% of cows treated with PGF$_{2\alpha}$ rut within a period of 36 to 130 h after administration (Whittier et al., 1989). Some protocols have been designed based on single or double doses of PGF$_{2\alpha}$ to reduce treatment costs. However, these protocols require estrus detection for a few days and/or the detection of a corpus luteum (CL) that is likely to be responsive to the treatment (CL of day 6 to 17 of the estrus cycle). One effective way to use the luteolytic effect of PGF$_{2\alpha}$ is to combine PGF$_{2\alpha}$ with progesterone in cycling cows. In general, females are treated with progesterone for 7 to 9 days and PGF$_{2\alpha}$ is administered 0 to 2 days before progesterone withdrawal.

### 2.1.2. Progesterone and Estradiol

The use of progesterone with estradiol controls the rise of follicular waves by promoting atresia of the developing follicles and regulates CL function and ovulation time. When combined with a luteolytic agent and an ovulation inductor, this treatment allows the use of AI at a defined time without estrus observation and with stable pregnancy rates. Estrus observation is not needed and is actually not recommended because FTAI systems frequently induce fertile ovulation without estrus expression (Thatcher et al., 2002). Such hormonal protocols can also increase ovarian activity. Progesterone and its synthetic analogues (progestagens) prevent the manifestation of heat because they mimic the endocrine activity of a CL. Progesterone supplementation is also an effective method for treating anestrus, which is a common problem in dairy production systems.

The removal of the progesterone is followed by estrus, ovulation, and a normal-length luteal phase in a large percentage of treated cows (Lucy et al., 2004). Currently, synthetic progestagens are best supplied in the form of subdermal ear implants; intravaginal implants are mostly used to release progesterone itself. The releasing devices vary in shape, design and the amount of hormone released. Estradiol suppresses the release of FSH, thus inducing the atresia of minor follicles. Progesterone decreases the release of LH, thus restricting the activity of larger follicles. It is therefore expected that all follicles in the ovaries reach atresia. It is also likely that a new follicular wave appears after 3 to 4 days of treatment with estradiol and progesterone (starting from any day of the estrus cycle).
Figure 1. Example of a fixed-time artificial insemination (FTAI) hormonal protocol used for lactating dairy cows. Estradiol benzoate is administered on a random day of the estrus cycle with a progesterone-releasing device; on day 8, the device is removed, together with a cloprostenol (a synthetic analogue of prostaglandin F2α) and eCG (equine chorionic gonadotropin) administration; 24 h later, a new dose of estradiol benzoate is administered; gonadorelin (synthetic analogue of GnRH - gonadotropin-releasing hormone) is given 24 h later; following 8 to 10 h, timed-AI is performed.

Additionally, estrogen is essential for the expression of oxytocin receptors in the endometrium which are critical to the release of PGF2α for CL regression. Therefore, estrogen has been used for both the synchronization of follicular waves and the promotion of luteolysis. Several drugs accomplish these purposes. Esters of estradiol, estradiol benzoate (EB) or estradiol cypionate (EC) have the lowest cost and are the most commonly used inductors in traditional protocols. Intervals to wave emergence depend on the dose and the half-life of the ester used. Hormones such as GnRH, LH and hCG may also be used as ovulation inducers, but their use is less common due to their higher costs. With the use of ovulation inductors, FTAI can be performed 52 h after the removal of progesterone, i.e., 8 to 12 h before the planned time of ovulation (Hanlon et al., 1997). An example of a FTAI protocol with the use of a progesterone-releasing device and estradiol is illustrated in Figure 1.

2.1.3. GnRH

GnRH is a hypothalamic hormone that acts by triggering the pituitary release of FSH and LH, thereby inducing ovulation. Despite having a short half-life in plasma (2 to 4 min), a single administration of GnRH results in an enhanced and simultaneous release of both LH and FSH in a log-dose response manner. In FTAI protocols, the ovulatory GnRH dose is typically given 48 h after a luteolytic dose of PGF2α. In countries where the use of certain drugs such as estrogen is not allowed, protocols with GnRH and PGF2α are highly convenient for FTAI in dairy cattle. Both hormones were of great importance for the consolidation of the FTAI protocol and the initiation of a series of other protocols. In addition, GnRH can effectively be used to restore the cycling of a female after calving because the ability of the pituitary to respond to GnRH is restored approximately 20 days postpartum.
2.1.4. eCG

Known as a mixed bioactive drug, eCG has been widely used in FTAI protocols. It can be employed both at the end of the treatment and at the emergence of the new wave because eCG is composed of 2/3 FSH and 1/3 LH. Souza et al. (2009) reported improvement in the fertility of dairy cows with low BCS treated with eCG on day 8 of the FTAI protocol. This effect was most likely due to an increase in activity of the ovulatory follicle (and consequently, of the CL) stimulated by this drug. High-producing milk cows have high levels of hepatic metabolism of ovarian steroids; the decrease in circulating progesterone during the luteal phase may therefore suppress early embryonic development. Treatment with eCG prior to ovulation can increase circulating progesterone levels and provide higher pregnancy rates in animals with low BCS.

2.2. Association of GnRH and PGF$_{2\alpha}$: Ovsynch

Based on a GnRH and PGF$_{2\alpha}$ combination, the Ovsynch program and its variations are the most commonly used protocols for FTAI on North American dairy farms. The first administration of GnRH occurs on a random day of the estrus cycle and stimulates the ovulation of the current dominant follicle. Ovulation is expected to happen within 2 or 3 days followed by the beginning of a new follicular wave. In order for the luteolytic process to occur, a PGF$_{2\alpha}$ injection is required; such an injection takes place 7 days after the first supply of GnRH. A second dose of GnRH is used 48 h after the PGF$_{2\alpha}$ administration to promote ovulation of the new DF. Ovulation then occurs in approximately 24 h, enabling the use of FTAI within 16 to 20 h (Pursley et al., 1995).

![Figure 2. Ovsynch is a fixed-time artificial insemination (FTAI) program that combines GnRH and PGF$_{2\alpha}$. GnRH is administered in a random day of the cycle; 7 days later, PGF$_{2\alpha}$; 48 h later, GnRH again; and AI 16 to 24 h after the last GnRH administration.](image-url)
The Ovsynch program is represented in Figure 2. It is possible that some females may not respond to the initial application of GnRH, which can occur in phases where there is no dominant follicle to ovulate, such as in the beginning or middle of the estrus cycle, and can disrupt the entire sequence of the protocol.

This is one of the critical problems associated with the Ovsynch program. Another important factor to consider is the number of follicular waves. The higher the number of waves, the less satisfactory the results will be. At the beginning of every follicular wave, there is a range of 4 days during which GnRH has no effect. When there are two follicular waves in the estrus cycle, there are two intervals of 4 days (the beginning of the waves). These periods where no response to GnRH can occur increase when the cycle has three waves, or three 4-day intervals, corresponding to 12 days in which the first application of the protocol will not be effective.

The number of waves is influenced by a number of factors, including the age of the cow. Two-wave cycles are more common in older cows than in heifers (Moreira et al., 2000). Diet can also influence the number of waves, as the type of feed provided can greatly influence the hepatic metabolism of steroid hormones. During digestion, a diet rich in grains requires greater hepatic activity, consequently generating a low serum concentration of steroid hormones such as progesterone. Lower levels of progesterone will lead to an increase in LH levels because less of this gonadotropin will be inhibited by progesterone. With higher levels of LH in the system, the DF present in the first wave of the cycle may remain available for longer, extending its wave duration and delaying the start of the next one. As a result, the third wave will not have time to occur because the hormonal profile at the end of the second wave will match that of the beginning of the third, thus culminating in ovulation.

This type of highly concentrated diet is typically given to high-producing cattle, explaining why these cows tend to have two follicular waves rather than three. This high metabolic clearance rate, leading to a reduction in the levels of circulating estradiol, can also result in a short duration of estrus, which explains why it is difficult to detect signs of estrus in dairy herds (Wiltbank et al., 2006). One approach for obtaining a better reproductive outcome in dairy cattle is to use three consecutive FTAI protocols and later relocate the cow for natural service by bulls. This allows the servicing of cows with several inseminations before transfer and typically results in a 15% greater chance of pregnancy. The resynchronization can be performed with either Ovsynch program protocols or with progesterone implants.
2.3. Important Considerations for FTAI In Dairy Herds

The use of FTAI protocols in dairy cattle is intended to decrease the common flaws that occur when relying on estrus observation and to optimize handling. However, the expected pregnancy rates with the use of hormonal protocols are similar to the ones obtained without the use of any drug when simply monitoring cows for signs of estrus. Therefore, the cost-benefit ratio and the pros and cons of the implementation of FTAI must be clear and rationally determined before using this tool. Most protocols of pharmacological control of estrus provide benefits, and major additions to reproductive efficiency can be expected when they are used in herds with adequate conditions.

Some dairy breeds are particularly sensitive to climatic variations. European breeds such as Holsteins and Jerseys suffer great thermal discomfort under conditions of high temperatures and high humidity. Thermal stress is very damaging to FTAI as gametes are sensitive to these conditions. Nevertheless, it is important to remember that the male is responsible for half of the process of fertilization; therefore, it is also necessary to rigorously analyze the quality of the semen.

3. EMBRYO TRANSFER IN DAIRY COWS

South America currently leads the world in both in vitro and in vivo bovine embryo production. Such a position is interesting when considering that the predominant racial type in South America is *Bos taurus indicus*, also known as Zebu, which is highly adapted to the tropics. Zebu cows naturally produce more oocytes than taurine breeds, which leads to a higher number of in vitro-produced (IVP) embryos and pregnancies. The larger number of pregnancies justifies, in many occasions, replacing the in vivo-production of embryos with the in vitro technique. This finding contributes to a large-scale commercial application of in vitro fertilization (IVF). By promoting a prior selection of donors and bulls, it is possible to achieve high levels of productivity; this usually results in lower costs, thus enabling the use of IVF on a commercial scale.

Despite the rapid development of the technique since its emergence in the late 1980s, until a few years ago, IVEP was only used as a last resort when traditional techniques failed. However, the high genetic gains provided by
Strategies to Improve the Reproductive Efficiency of Dairy Cattle

IVEP to the herds and the constant pregnancy rates achieved with this system have contributed to making this system a first-line option.

3.1. Embryo Transfer

Embryo transfer (ET) is a term generally used to define more than one biotechnological technique. It involves the production and transfer of embryos generated either in vivo by the technique of Multiple Ovulation and Embryo Transfer (MOET) or in vitro. In both cases, the purpose is to obtain genetically superior embryos and to generate pregnancies in female recipients. These biotechnologies aim to produce a much larger number of offspring than would be possible by natural methods of reproduction throughout the lifetime of a female.

An additional advantage provided by these biotechnologies is a better sanitary control of the reproductive process, especially with regard to diseases of the reproductive tract. In addition to preventing sexually transmitted diseases, the embryos can undergo sanitary procedures before the transfer, further minimizing the chance of transmission of pathogens. Embryo transfer can also be used to obtain descendants from donors that have acquired reproductive disorders or any other non-congenital condition that could prevent the female from producing and carrying healthy embryos. Thus, it is possible to prevent the premature discarding of genetically superior females that have acquired different types of reproductive disorders. Finally, these biotechnologies allow the performance of basic research on the physiology of reproduction and of surveys to assist the development of other biotechnologies such as cloning and transgenesis.

3.2. Selection of Donors

To be selected as embryo donors, females must have high levels of milk production. It is also very important to evaluate the reproductive performance of the donors; if their reproductive efficiency is good, the chances of getting the expected results with ET are higher. Donors must be free of infectious diseases such as brucellosis, tuberculosis, leucosis, leptospirosis, IBR and BVD.

Although some of these diseases can be controlled by treating the embryos before the transfer, the efficiency of embryo production in these animals may
be compromised by the infectious process. Donors with stressful conditions such as hoof or udder disorders generate poor results and should therefore be treated or allowed to recover from such situations before being included in ET programs.

3.3. Multiple Ovulation and Embryo Transfer (MOET)

This technique is divided into five steps:

- Selection of donors and recipients;
- Superovulation and artificial insemination of the donor;
- Preparation of recipients;
- Embryo collection;
- Evaluation and transfer of embryos.

Superovulation aims to stimulate the growth and ovulation of the highest possible number of follicles in a follicular wave. The administration of hormones with gonadotrophic activity prevents the phenomenon of dominance which would lead to atresia of many minor follicles. One of the biggest drawbacks of the technique is the high individual variability in the response to superovulation among the donors; an aggravating 20 to 30% of donors do not produce any transferable embryo. The choice of the hormonal protocol should be carefully evaluated, taking into consideration the breed, weight and production of donors, the condition of the property and the skill of the employees who will perform the work. An example of hormonal protocol used for superovulation is described in Table 1. In general, the embryo collection is performed seven days after the insemination of the donor. During this period, the embryos are in the uterine lumen and at the morula or blastocyst stage, the most suitable ones for transfer and cryopreservation. The collection method mostly used currently is uterine flushing via the cervix. The viable embryos that are found are categorized and transferred into the recipients via transcervical or surgical implantation. In general, it is possible to perform MOET in animals ranging in age from 10 months to 18 to 20 years. However, very young heifers exhibit highly variable ovarian responses. Furthermore, the size of the pelvis and the size and tortuosity of the cervix should be considered, as they could potentially hinder the necessary procedures and jeopardize the success of the collection. Senescent cows usually present with lower production levels, and production rates tend to decrease as animals age.
Table 1. Example of hormonal protocol used for the superovulation of bovine females

<table>
<thead>
<tr>
<th>Day</th>
<th>Time</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>D 0</td>
<td>a.m.</td>
<td>P4 Device Insertion + E2</td>
</tr>
<tr>
<td>D 4</td>
<td>a.m.</td>
<td>FSH (20% dose)</td>
</tr>
<tr>
<td></td>
<td>p.m.</td>
<td>FSH (20% dose)</td>
</tr>
<tr>
<td>D 5</td>
<td>a.m.</td>
<td>FSH (15% dose)</td>
</tr>
<tr>
<td></td>
<td>p.m.</td>
<td>FSH (15% dose)</td>
</tr>
<tr>
<td>D 6</td>
<td>a.m.</td>
<td>FSH (10% dose) + PGF2α</td>
</tr>
<tr>
<td></td>
<td>p.m.</td>
<td>FSH (10% dose) + P4 Device Removal</td>
</tr>
<tr>
<td>D 7</td>
<td>a.m.</td>
<td>FSH (5% dose)</td>
</tr>
<tr>
<td></td>
<td>p.m.</td>
<td>FSH (5% dose)</td>
</tr>
<tr>
<td>D 8</td>
<td>a.m.</td>
<td>Estrus observation</td>
</tr>
<tr>
<td></td>
<td>p.m.</td>
<td>A.I. (12 h after estrus)</td>
</tr>
<tr>
<td>D 9</td>
<td>a.m.</td>
<td>A.I. (24 h after estrus)</td>
</tr>
<tr>
<td>D 15</td>
<td>a.m.</td>
<td>Recipients evaluation</td>
</tr>
<tr>
<td></td>
<td>p.m.</td>
<td>Embryo collection + embryo transfer</td>
</tr>
</tbody>
</table>

Doses: estradiol benzoate (E2): 2 mg IM; prostaglandin (PGF2α): 2 mL IM. FSH doses vary according to breed, animal category and manufacturer.

3.4. In Vitro Embryo Production (IVEP)

IVEP aims to produce embryos in the laboratory using oocytes from ovaries of slaughtered or living cows. Oocytes and sperm are prepared and matured in vitro for the fertilization and subsequent embryo formation after a period of in vitro culture. The embryos are then transferred to properly prepared female recipients. In terms of genetic merit and sanitary aspects, the same criteria previously mentioned in this chapter should be applied. Specifically, the reproductive condition of females should be evaluated by ultrasonography at the exact time of oocyte retrieval. As a strategy for improving the cost/benefit ratio of IVEP, ideally only donors with a large number of follicles would be subjected to OPU. Although all visible follicles can be aspirated, there is a better retrieval of oocytes when the diameter of the dominant follicles is <4 mm (Seneda et al., 2001). The animals that are
subjected to follicular puncture should be evaluated periodically to control the appearance and severity of the injuries caused by the aspiration procedure. In general, there are few alterations in the vagina and cervix, and those that do occur are mostly transient irritations. Sequelae such as fibrosis and adhesions can occur in cows subjected to several sessions of follicular aspiration if the procedures were not properly performed (Gibbons et al., 1994). It is important to note that follicular aspiration is a technique that has pros and cons. The risk of sequelae exists and should be considered. The procedure must be performed with minimal damage so that the results outweigh the expected disadvantages.

3.4.1. Strategic Use of IVP Embryos in Dairy Farming

The large amount of oocytes and IVP embryos produced by Bos indicus females has made IVEP commercially very attractive in recent years. When the first commercial laboratories emerged, the demand was very limited. The interest from breeders was small and the expense of the services almost restricted their use to elite beef cattle producers. However, over the last six or seven years, dairy farmers have begun to make greater use of the technique of follicular aspiration and IVEP with the goal of rapidly multiplying the number of animals with high genetic potential. During this period, IVEP became a commercially viable alternative to improve pregnancy rates and speed up genetic improvement. However, until a few years ago, large-scale IVEP was limited by several drawbacks. One of them was the long distances between the laboratories and the properties where the recipients were held. In Brazil, for example, the large cattle herds are usually located in new areas of livestock production, such as the northern region. Oocyte donors and the laboratories which service them, on the other hand, are concentrated mainly in the south and southeast regions of the country, thousands of miles away from the farms with the recipient animals. The limited ability of IVP embryos to be cryopreserved greatly restricted the contact between production and transfer sites. Consequently, embryos that could not be transferred fresh were often discarded.

Recently, some of these problems have been overcome. In a large-scale IVEP program using exclusively sexed semen from bulls of Holstein and Gir breeds, embryos were transported over distances ranging from 800 to 2000 km to be transferred to the recipients (Pontes et al., 2010). Using portable incubators, the embryos were transported during the early stages of development. They began the development in the laboratory and were then transported for 2 to 5 days at different stages of development (day 2 to day 5; day 0 = day of IVF). At the end of transportation, embryos were re-evaluated
and loaded into straws to be transferred at morula or blastocyst stages. In this outsized project, 20,000 Girolando (Gir-Holstein crossbred animals) embryos were produced with female-sorted sperm. In just over a year, 8,000 Girolando heifers were produced from Holstein, Gir or Girolando donors. The possibility of transporting fresh IVP-embryos contributed to a satisfactory cost effectiveness of the IVEP process, with pregnancy rates of 36 to 40% and an overall average rate of 39%. In Holstein cows that typically show lower production of oocytes, it is also possible to obtain good results with follicular aspiration. The most effective strategy is to perform a pre-selection of females, assessing follicular populations with the use of an ultrasound. By using only the females with higher numbers of follicles, indices can be quite satisfactory. It is interesting to note that non-lactating cows may have high numbers of follicles and oocytes.

3.4.2. Sorted Sperm

In dairy cattle, the possibility of choosing the gender of the calf is extremely advantageous because male calves are not of economic interest. Some properties choose to produce embryos with unsorted sperm and determine the gender of embryos by biopsy and DNA analysis. Although this method is an accurate and sensitive technique, approximately 50% of the embryos are found to be male and discarded. In addition, pregnancy rates are considerably lower with biopsied embryos than with intact embryos (Hasler et al., 2002). The use of sorted sperm makes it possible for almost all of the embryos to be of the desired gender and of the same quality as embryos produced with conventional semen. However, the process of sperm sorting reduces sperm viability and pregnancy rates are not always satisfactory with artificial insemination (AI). For IVEP, on the other hand, semen subjected to this technique can successfully be used because this production system requires fewer viable sperm cells. The rates obtained currently in IVEP with sorted sperm are very encouraging. In the IVEP program described in the previous section of this chapter, the use of sorted sperm allowed a more rational use of embryo recipients. When unsorted sperm is used, twice as many recipients are required to achieve the desired number of products of the expected gender. Nevertheless, there were bull and ejaculate effects, showing the need for a rigorous evaluation and previous selection of the best batches of sperm to obtain more advantageous results (Pontes et al., 2010). Therefore, transportation of embryos over long distances and the use of sorted sperm are crucial ways to improve the efficiency of large-scale IVEP.
3.5. Heat Stress

In dairy farms located in regions with humid and hot summers such as Africa, India, South America, and the southern United States, pregnancy rates can be very low during the months of heat stress. Some properties even opt for extreme measures, and do not inseminate females in heat during the warmer months of the year. Exposure of the oocytes to increased body temperatures has been linked to the degeneration of thecal and granulosa cells and to low quality oocytes with lower rates of fertilization and conception (Chebel et al., 2008). Given the greater sensitivity of gametes and embryos to high temperatures in early stages of development, ET on day 7 after fertilization seems to be the most viable option. For this reason, ET has been used efficiently to increase pregnancy rates in dairy cattle in months and/or regions of higher average temperature (Hansen, 2007; Vasconcelos et al., 2011).

3.6. Recipients

Selection of recipients must be carefully conducted, as they represent a step of fundamental importance to the success of MOET or IVEP programs. Adequate recipients do not need to have superior genetic qualities, but they must present with an appropriate size (suitable for the breed of the fetus) and have good milk production and proven maternal ability to nurture the neonates properly. The health criteria applied to the recipients should be the same as those that are applied to the oocyte or embryo donors. To ensure the success of IVEP programs and to achieve better pregnancy rates, a large number of animals should be made available to allow a rigorous selection of the most suitable recipients. However, the high cost of maintaining these animals may be prohibitive and compromise the economic viability of the embryo industry. The possibility of transporting fresh embryos in vitro solved the major obstacle of large distances. It was originally cost-prohibitive to use recipients located near the laboratories because the availability of these animals was low. With the transport of IVP embryos over long distances, it became possible to use animals from the largest recipients commercial herds situated far away from the laboratories and establish IVEP on a commercial production scale.

Consequently, the use of large herds of embryo recipients resulted in changes in the pattern of females used. Informally, the best recipients were considered to be crossbred heifers, usually half-blood Nelore or European breeds, such as Simmental and Braunvieh, with some production capacity.
However, due to a limited supply and high demand of these animals, this type of recipient became costly and scarce. Particularly for large ET projects, it became cost-prohibitive to use this type of heifer as embryo recipient. A successful case in Brazil involved the use of recently calved beef cows (Nelore - *Bos indicus*) as embryo recipients. This category of females is the most numerous in South America, providing an adequate supply of recipients at a fair price. Contradicting previously held ideas, these females have provided acceptable pregnancy rates (approximately 40%), and more than 10,000 calves have been born to these cows (In Vitro Brasil Ltda, Mogi Mirim, SP; unpublished data). If animals have adequate nutritional and health statuses, the use of cows may indeed present certain advantages over heifers. In general, cows may have a higher recipient transferred-to-treated rate with FTET because they usually show better response to hormonal protocols and heifers often require a pre-synchronization. Cows usually have also been more exposed to pathogens and thus may have greater disease resistance and better quality colostrum. Finally, the frequency of dystocia tends to be higher in heifers than in cows. Until a few years ago, the importance of recipients was based mainly on their ability to respond to synchronization protocols and to maintain pregnancy. However, with current advances in epigenetics, it became evident that other factors, such as diet quality and maternal behavior, can interfere with gene expression of the calf. Despite the fact that the genetic sequence is established at the first cell division, the expression of genes may be altered due to environmental factors. The follicular ovarian reserve of the fetuses, for instance, can be hugely affected by the nutritional status of the recipient during pregnancy. As a result, a future donor may have compromised reproductive performance if the recipient underwent nutritional deprivation during pregnancy (Ireland et al., 2011). Similarly, it was shown that females with strongly maternal behavior influence DNA methylation and thus interfere with the expression of important genes for environmental adaptation (Weaver et al., 2004).

### 3.7. Fixed-Time Embryo Transfer (FTET)

Similarly to timed artificial insemination (TAI), FTET eliminates the need for heat detection, resulting in great advantages for the strategic management of the breeding of the herd. To solve problems related to heat detection and to increase the proportion of suitable recipients to receive embryos, several
hormonal protocols have been developed. An example of FTET protocol is described in Figure 3.

3.8. Advantages of Using Embryos Instead of AI

Unlike AI, the use of embryos results in relatively constant pregnancy rates throughout the year, including during periods of high temperatures. ET allows the enhancement of genetic gain. With the use of AI, the number of descendants of sires genetically evaluated for desirable characteristics can be multiplied.

Embryo transfer, in turn, also increases the number of descendants of females of high genetic merit, causing an even more significant impact on the improvement of a herd. Therefore, greater genetic gain and a higher genetic leap are achieved in each generation with ET than with the use of AI alone. Embryo transfer can also increase the reproductive efficiency of repeat breeder cows. These animals are defined as subfertile females that do not have any anatomical or infectious abnormalities yet require three or more services to become pregnant. ET increases conception rates of these animals to levels close to those of normally fertile breeder cows, suggesting that reproductive failures are associated with low oocyte quality and early embryonic developmental defects. ET can therefore be an effective method of preventing early embryonic mortality and increasing pregnancy rates.

Because of these advantages, some dairy farms have completely replaced AI with ET, achieving better pregnancy rates and accelerating genetic gain between generations. In these situations, recipients and donors are dairy cows from the same herd; the cows with the best genetic potential are used as oocyte or embryo donors and the remainders are used as recipients. Thus, efficient genetic selection is achieved from animals from the same herd.

Figure 3. Example of a hormonal protocol for fixed-time embryo transfer (FTET).
3.9. Advantages of Using In Vitro- Instead of In Vivo-Produced Embryos

When performing the in vivo production of embryos, it is necessary to superovulate the donor with the administration of hormones. With OPU/IVEP, oocytes can be obtained without the use of drugs and the sequential production of embryos can be performed without exogenous hormone stimulation. The in vitro technique also allows the use of a smaller interval between follicular aspirations in the donor. In general, a minimum interval of 15 to 30 days is used before the females are subjected to a new session of follicular aspiration. There is no established limit for the number of aspirations, and reports of up to 20 procedures performed sequentially without harm to the donor exist. Moreover, superovulation requires intervals of 40 to 60 days and can only be used approximately 3 to 4 times before a gap of several months is recommended. Perhaps one of the major advantages of IVEP is its efficient use of sexed semen. The sorting process often compromises the fertilization efficiency of a portion of the spermatozoa, making the process of in vivo fertilization more challenging. In the in vitro process, the sperm are subject to less demanding conditions, providing better results than AI and in vivo embryo production. Production of embryos by MOET does not allow the use of pregnant animals. In IVEP, it is possible to produce embryos by aspirating the ovary of pregnant animals as long as the aspiration can be performed without excessive pulling. Another advantage of IVEP is that it enables higher use of a fixed dose of semen: a single dose can be used to fertilize oocytes of 10 or more females.

3.10. Critical Aspects of the Use Of IVP Embryos:

The OPU/IVEP technique has more steps than the in vivo production technique. Thus, highly trained professionals are needed throughout the process to obtain satisfactory results. The amount of embryos produced can be decisive in the commercial viability of the technique. Because OPU and laboratory staff are associated with fixed costs, higher numbers of attempted pregnancies can result in lower overall costs. Both cryopreservation and rewarming processes are more critical in IVEP than in other techniques. Because they are structurally different from in vivo-generated embryos, IVP embryos cannot be subjected to conventional freezing without suffering severe damage. Currently, the most efficient method to cryopreserve IVP embryos is
vitrification. In this technique, no method of direct transfer exists yet, and vitrified embryos must go through a specific process of rewarming before being loaded and transferred. The genetic impact on a herd is higher with IVF than with other techniques. If the donors are selected properly, IVF can confer a significant improvement on each generation. On the other hand, any mistake in the selection of females can have a major negative impact on the herd. Hence, rigorous and objective criteria must be used in the selection of oocyte donors.

3.11. Cryopreservation and Strategic Use of the Embryo

To be cryopreserved, embryos generated in vivo are loaded into straws, in a similar manner to fresh embryos, with an additional cryoprotectant added to the medium. The most commonly used cryoprotectant is ethylene glycol, as it protects the blastomeres from damage caused by the low temperatures. Once they are filled, the straws are put in the freezing machine, which allows a controlled decrease of the temperature. When the temperature reaches approximately -7 °C, a "seeding" procedure, which aims to induce homogeneous crystallization of the contents of the straw, should be performed. After this procedure has been performed, the machine goes back to decreasing the temperature at a rate of approximately 0.3 to 0.5 °C per minute. This speed is calculated to prevent the formation of ice crystals within the embryo (if freezing occurs too rapidly) or the induction of cell damage due to the rapid dehydration of the embryo and hyper-saline concentration inside the cell (if freezing occurs too slowly). When the final temperature is reached (-32 to -35 °C), the straws are immersed in liquid nitrogen and stored in nitrogen tanks.

Unlike in vivo-generated embryos, IVP embryos are more susceptible to the damage caused by cryopreservation and rarely survive the freezing process. The greater sensitivity of IVP embryos to cooling appears to be directly related to higher lipid accumulation caused by the in vitro culture (Abe et al., 2002). These lipids can undergo irreversible changes and severely compromise embryonic development. A new method of increasing the cryotolerance of these embryos that has proven effective is chemical delipidation.

Forskolin, a substance derived from the Indian plant Coleus forskohlii, has been successfully used to promote intracellular lipolysis in embryonic cells (Men et al., 2006). When applied at strategic periods of the in vitro culture, this substance is able to increase the embryonic cryotolerance to levels that
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provide good pregnancy rates, enabling the commercial use of the technique. The pregnancy rates obtained by the laboratory In Vitro Brasil with vitrified embryos previously exposed to forskolin are described in Table 2 (unpublished data). With the advances in cryopreservation techniques of IVP embryos, it is possible to have a stock of female-sexed embryos for strategic use during the months of greatest heat.

**Table 2. Pregnancy rates of IVP Bos indicus embryos treated with forskolin for 48 h in culture and submitted to vitrification**

<table>
<thead>
<tr>
<th>Breed</th>
<th>Transferred embryos (n)</th>
<th>Preganacies (n)</th>
<th>Pregnancy rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>taurus-indicus</td>
<td>87</td>
<td>37</td>
<td>42.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gir</td>
<td>701</td>
<td>314</td>
<td>44.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Guzera</td>
<td>680</td>
<td>274</td>
<td>40.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nelore</td>
<td>440</td>
<td>200</td>
<td>45.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total</td>
<td>1,908</td>
<td>825</td>
<td>43.2</td>
</tr>
</tbody>
</table>

<sup>a</sup>Within a column, rates with a common superscript are similar (P > 0.05).

In addition to enabling better pregnancy rates than AI, IVP embryos have the added advantage of generating pregnancies of the desired gender. The use of IVP embryos in dairy farming has been the most promising feature of recent years, second only to the advances brought about by the pharmacological control of the estrous cycle and ovulation. Highly efficient technologies are already available, and large dairy farms have sought strategies to improve their herds using technology from biotech companies.

**CONCLUSION**

The failure to detect estrus in an accurate manner is one of the most common reasons for the failure of reproductive programs on dairy farms. Heat stress also represents a significant challenge, causing low pregnancy rates and therefore high economic losses. The technologies of FTAI and embryo transfer have directly benefited dairy farms of all sizes (micro, small, medium and large). However, the importance of factors such as nutrition and management must be considered before implementing these strategies because these factors directly influence reproductive efficiency. Any variation in these factors can
affect the result of a FTAI protocol or rates of embryo production. Thus, a proper analysis of the technique to be used combined with adequate conditions for the herd can provide optimal results.

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