Reproductive performance in high-producing dairy cows: Can we sustain it under current practice?

Rodriguez-Martinez H[1,2*], J Hultgren[2], R Båge[1], A-S Bergqvist[1], C Svensson[2], C Bergsten[2,5], L Lidfors[2], S Gunnarsson[2], B Algers[2], U Emanuelson[3], B Berglund[4], G Andersson[4], M Håård[5], B Lindhé[5], H Stålhammar[5] & H Gustafsson[6]

[1]Division of Reproduction, Dept of Clinical Sciences, Faculty of Veterinary Medicine and Animal Science (FVMAS), Swedish University of Agricultural Sciences (SLU), PO Box 7054, 75007 Uppsala, Sweden
[2]Dept of Animal Environment and Health, FVMAS, SLU, PO Box 234, 532 23 Skara, Sweden
[3]Division of Ruminant Diseases and Epidemiology, Dept of Clinical Sciences, FVMAS, SLU, Uppsala, Sweden
[4]Dept of Animal Breeding and Genetics, FVMAS, SLU, PO Box 7023, 75007 Uppsala, Sweden
[5]VikingGenetics, Örnsro, PO Box 64, SE-532 21 Skara, Sweden
[6]Swedish Dairy Association, PO Box 210, SE-101 24 Stockholm, Sweden
* Corresponding author: heriberto.rodriguez@kv.slu.se

Summary

Milk yields >10,000 kg/year are common in modern dairy production, owing to improved nutrition, management and genetic gains through use of progeny-tested bulls. However, reproductive performance has decreased worldwide in many cows with a high genetic potential for milk production, particularly in the Holstein breed. Moreover, cow robustness and longevity is also threatened by increasing stress, udder health disturbances and of locomotion disorders. Genetic global misuse of a narrow base of AI sires -including those selected for high milk yield but not consequently for health and reproductive traits- has not only contributed to these undesirable effects on animal health and welfare but, together with sub-optimal management, jeopardized the ethical and economical sustainability of modern dairy farming. This review describes the state-of-the-art of this multifaceted problem and advises on how to ameliorate it, since it is not seen as an unsolvable problem. Use of high-fertility sires, of balanced breeding programs with adequate trait measurements, diet optimization, design of buildings and management systems that best support reproduction as well as cross-breeding; are among short- and medium-term strategies. In a longer perspective, holistic- and trait-orientated research on interrelations between gene regulation of nutrition, lactation and stress is needed; aiming at identifying reliable and cheap markers to be used on-line and on-farm as recorders of genetic traits. Awaiting the full application of juvenile genomic selection, a wider inclusion of functional traits (fertility, health and longevity) and of product quality are mandatory for breeding programs in order to secure acceptable fertility, sustained milk production and the best welfare of dairy cows. Such strategies have proven successful in the Nordic countries and are being increasingly adopted by others.

Introduction

Milk yield of the dairy cow has increased rapidly over the past 40 years and, in some European countries more than doubled, due to a combination of improved genetics, feeding and management, with an overwhelming focus on milk production volumes. ***Figure 1*** depicts recent (years 2003 to 2008) global trends in yearly milk yield/cow in selected countries/regions. Sweden is leading the European Union (EU) milk yield league with national averages of 9,718 kg of Energy Corrected Milk (ECM)/lactation for Swedish Holsteins (SH) and 9,164 kg for Swedish Red cows (SR) (2006/20007) (Swedish Dairy Association 2008, http://www.svenskmjolk.se) (see ***Figure 2***). Overall, the genetic gain in milk production reaches 1.5% per year, mostly owing to the effective use of artificial insemination (AI), progeny testing, and intense selection of bulls for world-wide use. However, this high milk productivity has been shadowed by a documented global decrease in average dairy herd reproductive performance (e.g., the ability of the female to produce a live calf), particularly for the dominating Holstein breed [1] (see trends for selected countries in ***Figure 3***). Fertility, a component of reproductive performance defines the ability of the female to become pregnant, but it is -at the end- reflected in the birth of a calf. Fertility is usually monitored by indirect (and sometimes erratic) rates of non-return to estrus (NRRs) or by the more accurate conception (CRs) or pregnancy rates (PRs), resulting from clinical examinations. Interestingly, while fertilization rates in dairy cattle can be as high as 85–90%, and CRs are probably above 70% [2,3], calving rates in Holsteins are below 40% in most cases [4] and in some reports as low as 25% [1,5], decreasing at rates of 0.5–1.0 % units/year in American Holstein, a problem that was detected already in the late 1950´s [6,7]. Such a decline in calving rates is, however, not observed in Holstein heifers [8]. Reproductive performance, being affected by fertility, embryonic and fetal development, and ultimately by calf survival; is monitored by indicators such as the interval between calving and successful breeding (number of days open, DO) or the interval between successive calvings (CI). Both these indicators are affected by bull and cow fertility and by herd management factors, such as estrous detection, number of AIs per conception, and the interval between calving and time to the first AI. ***Figure 4*** **A-B** clearly illustrates trends of increased numbers of DO, of services per conception and of extended CIs in American Holstein cows, over the past 25 to 35 years, respectively. These are indicators of a decreasing reproductive performance which, increasing culling, adds significant costs to an already constrained milk production sector.

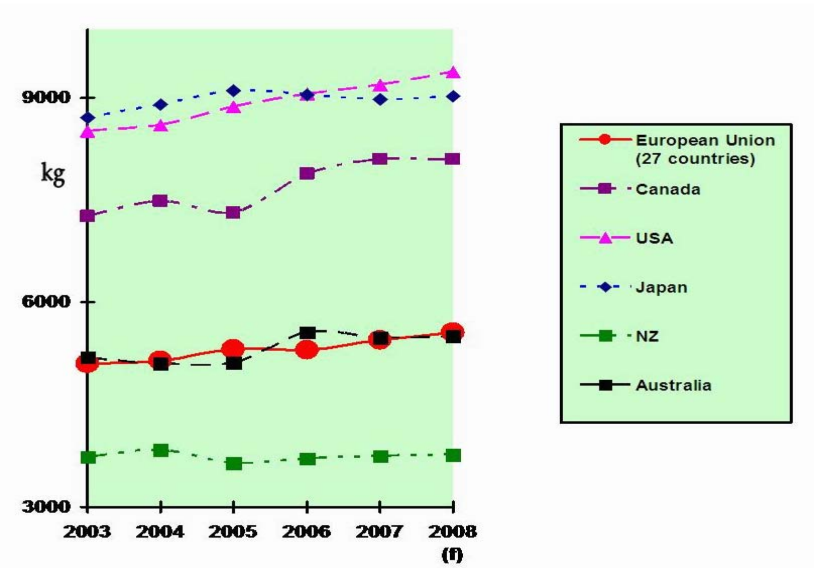

Figure 1: Global trends in milk yield per cow-year (kg) in selected countries/regions for the period 2003–2008 (f=forecast) (Source: www.fas.usda.gov/dlp/circular/2007/dairy_12-2007.pdf).

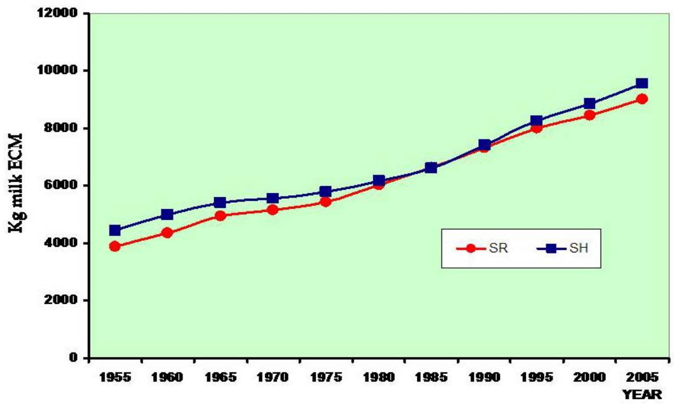

Figure 2**:** Average annual milk production (kg energy-corrected milk, ECM) for cows of the Swedish Red (SR) and Swedish Holstein (SH) breeds, period 1955–2005 (Source: Swedish Dairy Association 2008, http://www.svenskmjolk.se).

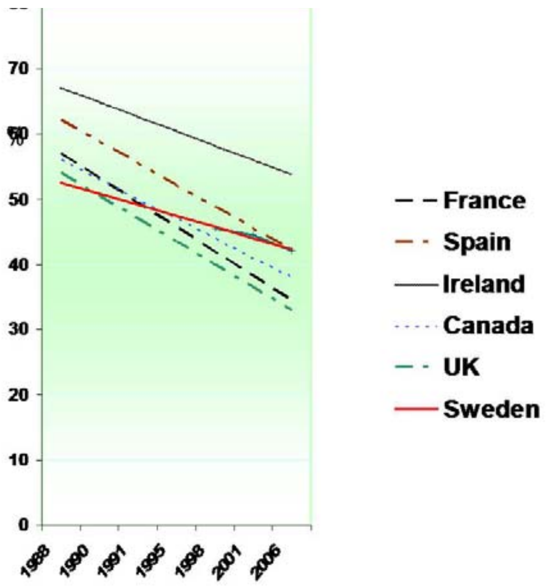

Figure 3**:** Trends of fertility decrease in Holstein cattle in selected countries between 1988 and 2006. Note the more horizontal slope for the Swedish population, compared to the other countries (Source: literature review, present paper).

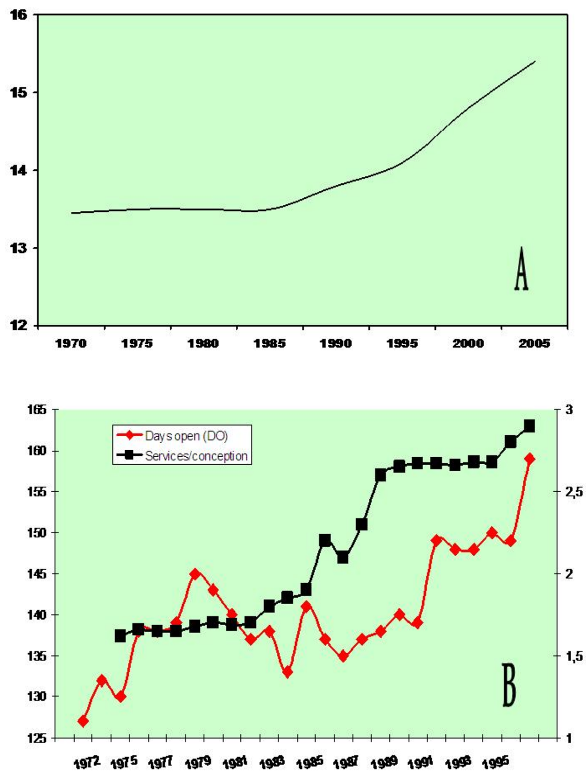

Figure 4: (A) Average calving interval (CI, months) in American Holstein cows for the period 1970–2005 (Source: Oltenacu & Algers, Ambio 34, 2005, http://www.ambio.kva.se, modified); (B) Changes in days open (DO) and services per conception in 73 Holstein herds in Kentucky, USA 1972–1996 (modified from Silvia WJ, J Dairy Sci 81, Suppl 1, 2003, with permission).

Fertility is one of the most complex measures of reproduction, being undisputably influenced by genes and environment. However, although these two components act in concert, they synergistically mask the contribution of the other thus confounding selection strategies for fertility and, ultimately, affecting reproductive performance [9,10,11,12]. As already mentioned, milk yield-focused selection has been tradition in dairy. Unfortunately, there are clear negative genetic relationships (several times stronger than the phenotypic ones [13,14]) between milk yield with fertility, presence of mastitis and other health traits. Surprisingly, large genetic variation is found in these fitness- and welfare-related traits, despite their low heritability [11]. The unfavourable correlations found were mostly ~0.2–0.4, indicating that selection for milk yield alone would lead to poorer fertility and animal health, thus seriously affecting cow welfare [15,16]. However, as the correlations are far from unity, they also indicate that there are individual cows which can combine a high yield with good health and fertility, as has been seen in Sweden [17], provided the cow has inherited good genes for both milk production and fertility and/or that management is of such quality that it can compensate for the negative genetic effect. Unfortunately, such compensatory management is hard to apply in large herds (a product of the structural change suffered by the the dairy production sector), where high-producing cows, e.g., with >11,000 yearly kg milk yield are most often seen, unless proper buildings, enough well-trained personnel with time to work with the animals are present. The increasing use of do-it-yourself-AI (DIY-AI) has been associated to increasing costs. In Sweden, where ~40% of the AIs are performed by the farmers themselves (the highest figure in Scandinavia), the DIY-AI fertility is ~3-4% lower than that reached by AI-technicians (Swedish Dairy Association 2008). Less time available for estrous detection is among the reasons, but also weaker display of estrous signs has been recorded.

In any case, at a global scale, most modern dairy AI-bulls have been primarily selected for the single trait of milk yield, with dramatic increases in genetic gain but accompanied with a decline in reproductive performance, probably by the insufficient correlation between these two traits. Most dairy cows, particularly those high-yielding, suffer of a negative energy balance (NEB) period during the first weeks of lactation, when energy output in milk exceeds energy intake from the diet. Such NEB is associated with a multi-factorial syndrome of sub-fertility during this period, when ovarian function (monitored as estrus and ovulation) is to be re-established to warrant a new pregnancy, the prerequisite for a renewed lactation. Moreover, the increasing use of unbalanced breeding in particularly the Holstein breed has caused declining calving rates over the past decades in countries that had heavily used this breed [1]. A series of questions are therefore presented, involving genetic selection, nutrition and management, for instance: Has the potential of cows to consume man-prepared feedstuffs for ruminants (concentrates, etc), often combined with some feeding strategies (such as the use of Total Mixed Ration, TMR) increased in pace with their production? Do all high-yielding cows suffer from serious metabolic stress (MS) and NEB, serious enough to dramatically impair their fertility? Has the use of exogenous hormones caused inhibition of estrus signalling? Is a constrained estrus signalling the major problem? When do reproductive losses occur? To what extent does the male, through epigenetic mechanisms, contribute to these losses? Should milk production be lowered in order to increase fertility? Evidently, considering the latter, there is also a need for well-designed bioethical studies to enable a constructive societal debate regarding sustainable milk production. Additionally, we need to determine the biological mechanisms behind the relationships between productivity, animal health and welfare. Most importantly, we need to know how we can best measure and correct the current misfit, at the lowest possible cost, to the highest possible accuracy and in a long-lasting perspective. Obviously, understanding the reasons behind and devising proper solutions for such multifactorial problems require integration of many disciplines, including genetics, housing, management, nutrition, immunology, molecular biology, endocrinology, metabolic and reproductive physiology, ethology, and animal welfare [18].

The present paper reviews the state-of-the-art of the multifaceted problem of infertility and decreased reproductive performance in high-producing dairy cattle and suggest short-, medium- and long-term solutions available to ameliorate it or aid to its permanent solution.

Is selection for high yield the major cause of this problem?

Since genetic merit for milk production is generally recognized as the single most important objective in breeding programs for dairy cattle (mostly considering the low heritability of most common fertility traits, usually below 5%, owing to environment pressure), focus in breeding dairy has been primarily put, worldwide, on milk yield (but, in some countries, also on milk quality). As a result, in breeds such as the American Holstein, the genetic potential for milk production has increased by over 3,000 kg per lactation, and doubled over a 40-year period (***Figure 5***). A broader illustration of how sucessful a focused genetic selection of AI-bulls for higher milk production can be is given in ***Figure 6***, which depicts the milk index for Holstein bulls ranked by Interbull (International Bull Evaluation Service, www.interbull.org) in a series of countries. Since the efficient transmission of this production trait to the progeny requires a high rate of reproduction, preferably via AI; low-fertility cows or AI with semen from low-fertile bulls will eventually result in poor CRs and hence limit the genetic and economic progress. In other words, fertility is most relevant to the dairy industry.

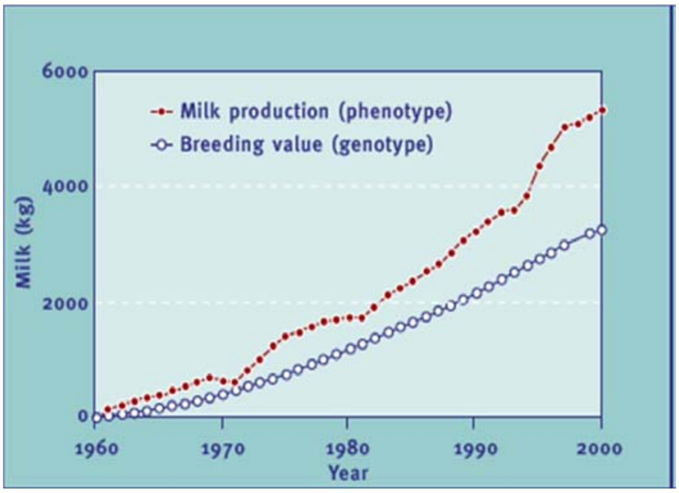

Figure 5: Trends of the genetic merit (breeding value) of AI bulls and actual average milk production of Holstein cows in the United States (Source: Silvia WJ, 2003, http://www.vetsite.org/publish/articles/000043/article.html, with permission).

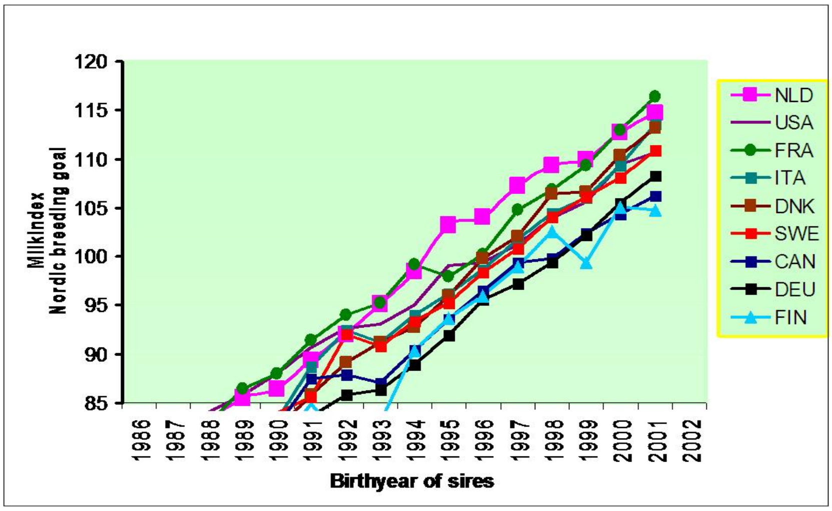

Figure 6: Genetic trend (milk index) for Holstein bulls in various countries ranked by Interbull August 2006, Nordic base and scale (source: Swedish Dairy Association, http://www.svenskmjolk.se).

Healthy, progeny-tested élite bulls are capable of producing well over 100,000 straws/year of frozen semen. Thus, there is need for only a relatively small number (<1,000 if all cows in the world were to be covered within one year!) of proven bull sires to service the world's population of dairy cows. A steady increase of exports of the North American Holstein from the 1970’s into the 1990’s (because of its perceived superiority regarding milk yield and other dairy traits compared with European lines) has led to a globalization of dairy genetics with bulls that are closely related, resulting in increasing inbreeding levels [19,20].

Most geneticists agree that inappropriate use of sires, selected without taking enough consideration to reproductive traits and focusing mainly for increased milk production, in countries that heavily used american Holsteins, has led a documented decline in reproduction success [1,11,21]. In some examples, the impairment of fertility and health has reached such an extent that it is now being considered a major obstacle for milk production management [19]. In the UK, for instance, pregnancy rate to first service has dropped from 56% in 1972-1982 to about 40% in 1995-1998, a rate of about 1% per year [4](see ***Figure 3***). This phenotypic trend is probably mainly caused by genetic deterioration in this breed since the problem is still there, despite having included reproductive performance both in the breeding goal and as selection criterion [22]. Using mathematical mechanistic modelling, UK researchers have prognosed that the current trend would lead the national dairy herd to be unsustainable due to increasing calving intervals and reduced fertility in as few as 10 years [23]. A yet undisclosed chapter is the possible presence of aberrant paternal mRNA in the spermatozoa which might cause epigenetic-like defects, visible during either early embryonic development, placental development or later fetal development [24], as we shall discuss later.

Actions have been taken to counteract these undesirable effects. The industry has used genetic markers to track recessive genetic disorders (qualitative traits) but the impact of tracking quantitative traits is yet limited [21]. Interbull has, over the last decade, launched international genetic evaluations for female fertility, mastitis and longevity, aiming at a fair comparison of bulls for non-production traits, both for Holstein and other main breeds of the Interbull member countries. However, comparisons are not yet global, and there are still many other bulls marketed, which are yet to be included in the breeding selection schemes promoted by Interbull. Fortunately, the emphasis of selection continues anyway to change from production to non-production traits, especially towards health and robustness, in an increasing number of countries, albeit with a large variation in their relative weight as selection indices (***Figure 7***) [25]. However, because this breeding selection is relatively slow, other measures have been taken to ameliorate poor fertility, the major breeding and management issue faced today by dairy farmers. For instance, crossbreeding has become mode, capitalizing on heterosis and the incorporation of breeds where selection has already gained from an important consideration for fertility (and other health traits) in the breeding goal [20].

Probably, the most important and long-lasting action has been demonstrated by the Scandinavian/Nordic breeders. They have obviously selected for increased milk yield. ***Figure 8*** depicts such increasing trend for Holstein Swedish (SH) bulls (both for unproven bulls, proven bulls and imported semen of different sources), which has been accompanied by a decrease in daughter fertility (***Figure 9***), confirming that genetically-driven milk production in dairy cows is clearly negatively associated with fertility [21,26,27] and with increased prevalence of mastitis [28,29]. There are, however, clear breed differences. The bull breeding values for daughter fertility within the SH breed dropped with more than two standard deviations over the past 20 years (***Figure 10***). Twenty years ago, 50% of SH bulls had a breeding value equal to the mean or better. Today, less than 2% of the bulls reach the same quality. Moreover, while the SH has shown a decrease in fertility or reproductive performance at a rate of ~1.2 index units/year (~0.25% units/year) until 2007 [17], the Swedish Red breed

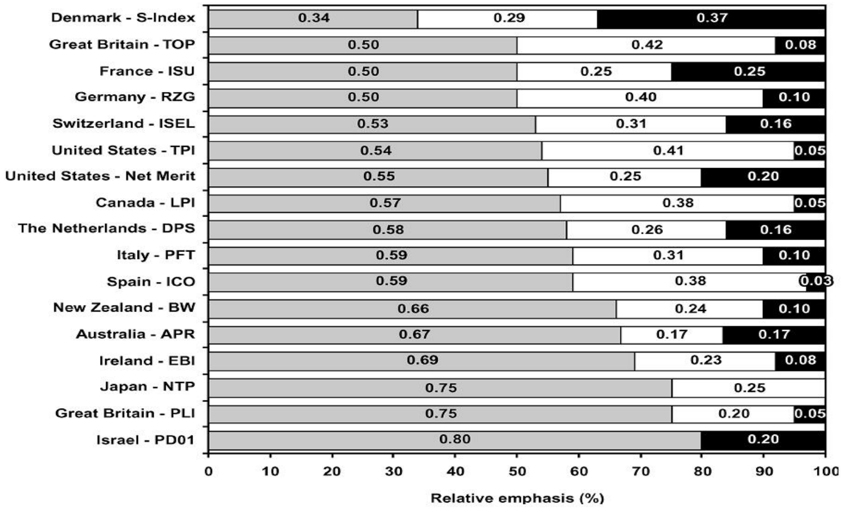

Figure 7: Relative proportions (%) of components of breeding selection indices for production (gray), longevity (white), and health and reproduction (black) in selected countries, August 2003 (Source: Miglior et al, J Dairy Sci 2005; 88, with permission).

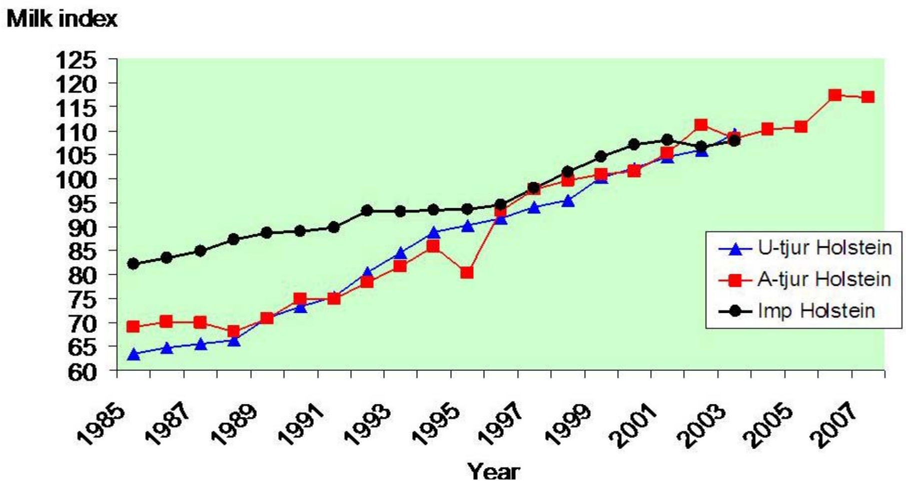

Figure 8: Milk index for AI Holstein sires in Sweden, as young (U), progeny-tested (A) or imported (Imp) for the period 1985-2007 (Source: http://www.vikinggenetics.com).

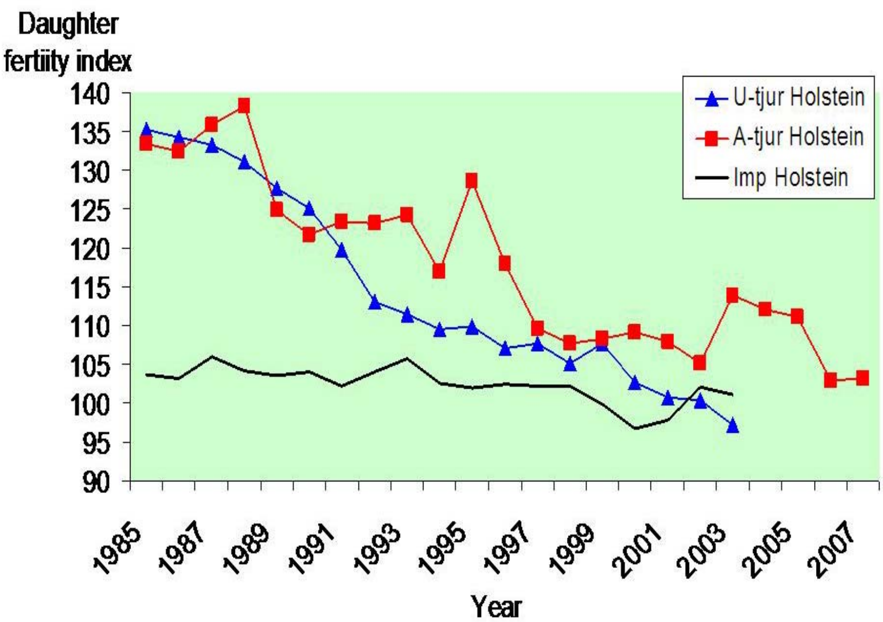

Figure 9: Daughter fertility index for AI Holstein sires in Sweden, as young (U), progeny-tested (A) or imported (Imp) for the period 1985-2007 (Source: http://www.vikinggenetics.com).

(SR) has maintained fertility over the same period, despite also having a high milk production potential and similar milk yield (see ***Figure 2*** for average annual milk production for SR and SH breeds since 1955, and ***Figure 10*** for the trends in conception rates for the two breeds since 1998).

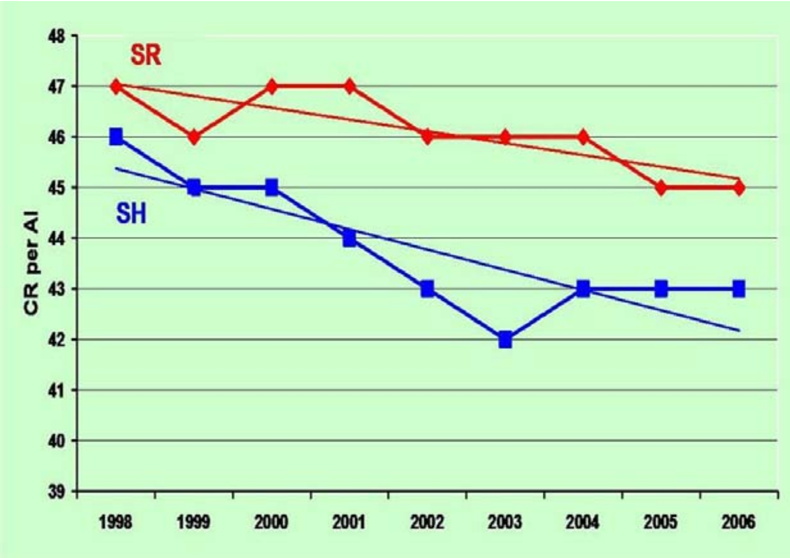

Figure 10: Conception rates (CR, including trend lines) per AI in dairy cattle of the Swedish Red (SR) and Swedish Holstein (SH) breeds, period 1998–2006, where fertility and health traits are included in the breeding goal. (Source: Swedish Dairy Association 2008, http://www.svenskmjolk.se).

Overall, in comparison with other countries/regions, Sweden has been able to prevent the decline of daughter fertility (see ***Figure 3***) and to stabilize the resistance to mastitis by using a sound Swedish national breeding program and the direct incorporation of several reproducion and health functional traits (such as fertility, longevity, calving ease, udder health and claw health) for more than 30 years [25,30,31]. The rationale behind has been that despite the low heritability of fertility and health traits, there is yet a significant genetic variation and thus a chance to balance the genetic gain for production and animal health and reproduction. Data in Sweden are available for all AI sires and for ~86% of the females in milk recording, thus making the system an outstanding tool for research, particularly for their linkage to underlying animal welfare mechanisms, upon which selection ought to be based. In sum, despite the obvious presence of complex physiological associations that most likely rule a negative genetic correlation between milk yield and fertility [32], such reduced fertility would not need to be a direct consequence of the genetically-driven increase in milk yield but rather a lack of proper selection weight for fertility traits in the breeding goal [33].

Management and nutrition also play important roles, considering the association between milk production and fertility varies between herds, both genotypically and phenotypically [34,35]. Selection for increased milk-yield decreases the amount of total energy consumed that is used for maintenance, because such energy is -instead- used for the formation of milk, causing a genetically-induced NEB and a poorer body condition (BC), both physiological states directly linked to a decreased fertility. The so-called “expendable” processes (e.g. fat storage and reproduction) are the first to be down-regulated during nutrient deficiency or imbalance, while lactation, thermoregulation, growth and other “reducible” processes are maintained unless the nutritional status worsen [36]. An aggravating detail is that although selection for milk yield partly increases feed intake (genetic correlation 0.45-0.65 [32]), it does not improve feed efficiency. Instead, it increases live weight (LW) and the proportion of metabolically active organs such as the gut or the liver due to a positive phenotypic correlation, ultimately leading to a dramatic increase in maintenance requirements. Maintenance requirements of a high producing Holstein dairy cow in the 21st century is >25% higher than 30 years ago [37] following the changes in conformation (to a more angular cow) and size of the animals within the breed (see ***Figure 11***).

Figure 11**:** Changes in body conformation in American Holstein cows (upper) and bulls (below) during the past 40 years (Sources: K Wiegel and B Lindhé, 2007).

Taken together, selection for milk yield has increased the gap between energy input and output during early lactation, leading to a deepened NEB and MS. The dilemma of genetically-driven milk yield improvement and MS raises important challenges for sustainability of the dairy industry. It clearly influences the ability of the cow to promptly resume ovulation postpartum, the success of fertilization and the ability of the conceptus to proceed with pregnancy (see below). However, the large individual differences observed in the magnitude of NEB, even among cows with similar milk yields, indicate that opportunity exists to meet this challenge, provided we can understand better the consequences of selection for increased milk yield on feed intake, NEB and on major welfare indicators, such as animal behaviour, health and fertility. Once again, Scandinavian data are clear on this point; increasing the weight for fertility traits in selection has halted the negative genetic decline while maintaining a large proportion of the yearly increase in milk yield, as shown in ***Figures 2***, ***3*** and ***6*** [17]. However, there is still much to be done in relation to, for instance, estrus signalling. The duration of estrus has -globally- become shorter over time (***Table 1***), presumably in direct relation to an increasing milk yield (see ***Figure 12***).

Table 1: Changes in the mean duration of estrus (h) in dairy cows and heifers – published data**.**

Reference	Cow	Heifer
Hammond 1927	19.3	16.1
Trimberger 1948	17.8	15.3
Dransfield et al. 1998	7.1	-
Nebel & Jones 2002	10.8	-
Båge et al. 2002	-	15.2

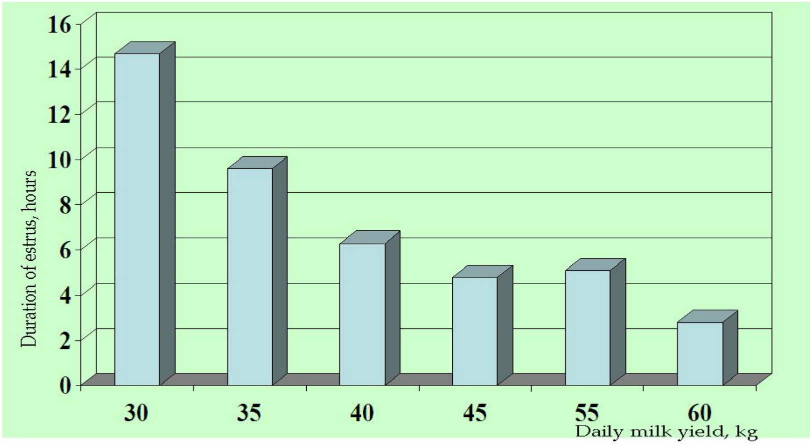

Figure 12**:** Duration of standing estrus (h) in American Holstein cows with different milk yield (Source: Lopez et al, Anim Reprod Sci 2004; 81, with permission).

Metabolic load during lactation, health and welfare in high-producing dairy cows

In dairy cattle, metabolic load (ML) is defined as "the total energy burden imposed by the synthesis and secretion of milk, which is met by mobilization of body reserves", while MS is defined as "the amount of ML that can not be sustained by this mobilization, leading to the down-regulation of some energetic processes, including those that maintain general health" [38]. In high-genetic-merit cows, selected for high milk yield, there is an increased body-tissue mobilization and an increased ML [39] which, in some of these cows, can be reflected by a period of marked NEB during early lactation, compared to cows of average genetic merit [9]. Cows in NEB down-regulate their own protein synthesis owing to a shortage of amino acids from the diet, while they mobilize their tissue fat to maintain, or even increase, the secretion of milk fat. Energy balance begins to decrease during the last few weeks prior to calving primarily due to a 30-35% reduction in voluntary feed intake. Moreover, after calving, the mobilization of body reserves and the volume of milk secretion increase much faster than the voluntary feed intake. Cows typically remain in NEB for 5–7 weeks postpartum [40]. Typical energy curves for a high-producing dairy cow are depicted in ***Figure 13***, showing the association of lactation and energy demands of the postpartum dairy cow.

The mobilization of body reserves during the period of lactational NEB is a key factor for disease susceptibility in dairy cattle [41]. The ability to store reserves, as subcutaneous fat, differs. For instance, Holstein cows have a thinner layer of subcutaneous fat compared to Swedish Red [42]. The NEB relates negatively to metabolic and locomotor disorders, such as milk fever, ketosis and laminitis [43]. Because high-producing dairy cows, in order to maintain a good body condition (BC), have to spend a large part of the day and night eating and ruminating instead of performing other behaviours like grooming, exploring, interacting socially, or displaying sexual behaviour [44], NEB also affects behaviour negatively. When cows perceive their situation as stressful and they can not cope with it, abnormal behaviour follows. Cows with an active coping pattern display tongue-rolling, head-leaning, muzzle-pressing and self- or inter-suckling, whereas cows with a passive coping pattern become more inactive, which could cause a reduction in feed intake [45].

High milk production *per se* does not always elicit negative effects on health and fertility traits, and the effect seems to depend on the *farm and production environment* [35]. Relationships of housing, management and nutrition with fertility are described in ***Figure 14*** and a summary of the influence of some management factors in modern dairy is given in ***Table 2***.

The following management procedures influence fertility:	
• Total mixed ration (TMR)	-
• Tie-stalls	-
• Size (and breed, Holstein)	-
• Do-it-yourself AI	-
• Net gain results	+
• Automatic Milking System	+
• Ecological production system	+

Table 2: Management influence (positive or negative) on dairy cattle fertility. Swedish data including 2708 farms with >45 cows incorporated in the national cow monitoring system between September 2004 and August (Adapted from Löf et al. J Dairy Sci 2007: 90).

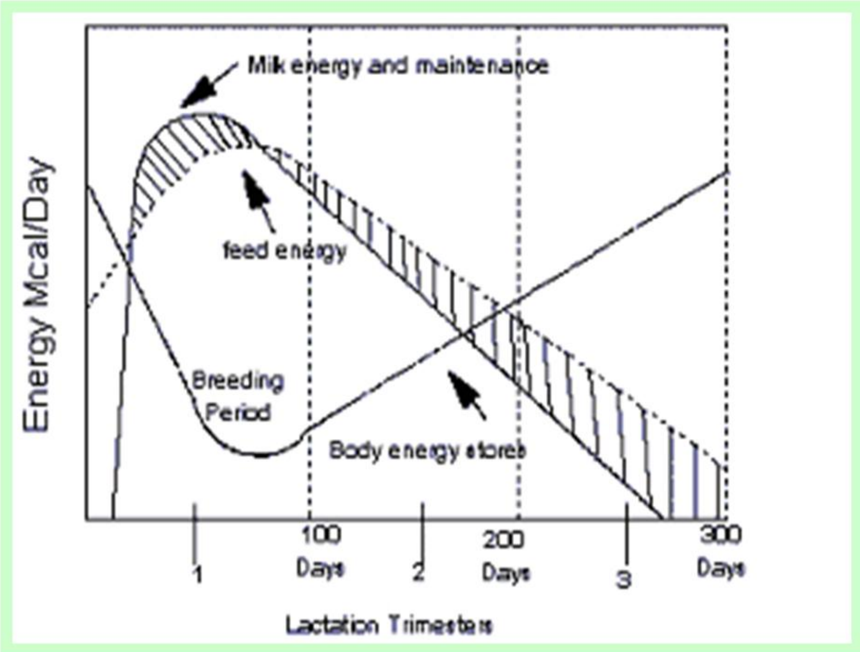

***Figure 13*:** Energy (in Mcal/day) curves (feed, milk production and body stores) for a lactating, high-producing dairy cow, in relation to time in lactation (trimesters) and breeding period (Source: Kutches A, Anim Nutr Health, Nov–Dec 1983).

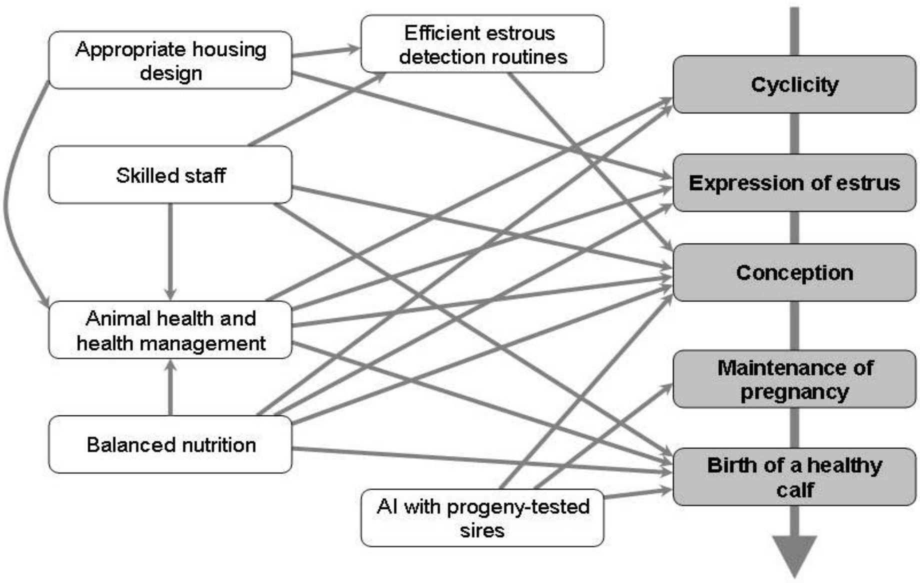

***Figure 14*:** Relationships of housing, management and nutrition with dairy cow fertility.

Actually, increases in herd size and use of loose-housing systems, while promoting the expression of estrous signs [12] and advancing the interval to 1st ovulatory estrus (***Table 3***), have led to increased incidences of some production-related diseases, including lameness, which has reached >20% incidence risk during lactation [46]. Some of the several causes for lameness relate to ML. For instance, and probably owing to stress-driven endocrine modifications, lame cows have reduced milk yield, decreased reproductive performance, and an increased risk of being culled [15,46,47,48,49,50]. The incidence of sole ulcers, an important cause of clinical lameness, is influenced by dietary levels, floor type, reproductive stage, cow conformation and genetics (reviewed by [51] and clearly afects reproductive performance [52], udder health, milk yield, and culling [15]. This calls for an increased detection of early lameness by e.g., automated kinematic analysis [53] and the use of better flooring systems (soft floors with friction). The latter not only prevent claw and leg problems but also enhances proper signalling of estrus and other behaviour.

Table 3: Effect of housing on the onset of postpartum (p.p) estrus in dairy cattle (Adapted from Pettersson et al, J Dairy Sci 2006; 89).

Housing	1st progesterone rise p.p. (d)	1st ovulatory estrus p.p. (d)
Tied	36.7	67.6
Loose	29.1	57.0

The environment should also be conducive to high voluntary feed intake, taking into consideration that cows with higher LW at lactation (usually those of higher milk yields) are supposed to be able to eat as much as they can, although their appetite and the size of their rumen are limited. As mentioned below, cows that are fat (high BC score) at calving usually eat less during lactation and sink into a deeper NEB, marking the importance of applying feed restrictions during the dry period. Cows need time and space for undisturbed feeding and rumination and react negatively if they need to compete for feeding space. Convincing evidence reveals now that the design of food passages, barriers and water troughs, as well as cow traffic within the building, affect the voluntary intake level of cows [54]. Transfer and re-grouping of cows challenge their social behaviour, and the energy spent to establish a new social hierarchy is no longer available to produce, or reproduce [55]. In accordance with the resource allocation theory [56], the animals´ resources are optimally allocated to different biological processes to maximise the animals´ fitness. Animals that have been selected for high production, including the dairy cow, may reallocate more of their resources into production traits and as a result to use less energy demanding behaviours which will reduce the animals´ capacity to cope with stressors such as the establishment of a social hierarchy or adapting to an unpredicable environment [57]. It seems obvious that a plethora of linked biological events mediate the unfavourable consequences of selection for increased milk production on the welfare of high-producing dairy cows, as outlined in ***Figure 15*** [16].

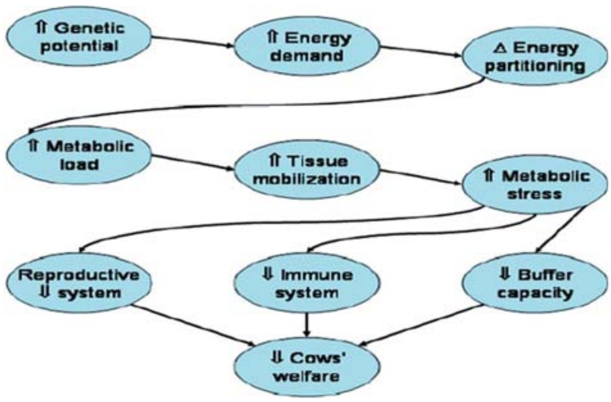

Figure 15: Interactions between biological events mediate the unfavourable consequences of aggressive selection for milk production on the welfare of high-producing dairy cows (Source: Oltenacu & Algers, Ambio 34, 2005, http://www.ambio.kva.se, with permission).

Why is the cow sub-fertile during lactation? Interaction between genetic selection for milk production and management

Studies on reproductive data from Holstein heifers have indicated that their CRs are significantly higher compared with 1st-lactation cows, marking the antagonistic relationship between lactation and reproduction [8]. Lactational NEB compromises fertility [58] because the time needed to recover from NEB acts as a metabolic modulator (restrainer) of the initiation of postpartum ovarian activity and of the behavioural display of estrous signs [59]. The high-producing cows of today have shorter estrous cycles, display fewer standing estruses, show estrus of shorter duration (***Table 1*** and ***Figure 12***), and often present double- or multiple ovulations. In the worst-case scenario, they are simply anestrous. Apparently, the reduced expression of insulin-growth factor binding protein-2 (IGFBP-2) mRNA in severe NEB cows might alter the bioavailability of circulating insulin-like growth factor (IGF-I) and of locally-produced IGF-II to modulate the pre-recruitment stages of follicles required to maintain normal postpartum ovarian cyclicity [60]. Moreover, NEFA released from the adipose tissue during NEB accumulates in the follicular fluid and disturbs follicle development [61,62]. Plasma IGF-I concentration in early lactation is, alongside with milk protein content, a useful indicator of reproductive efficiency [63]. ***Table 4*** summarizes some of the metabolic, endocrine and functional/clinical reproductive changes occurring as a consequence of ML and of NEB in high-producing dairy cows.

Table 4: Metabolic load (ML) causes negative energy balance (NEB) and increases metabolic rates impairing reproduction in lactating, high-producing dairy cows.

Metabolic load (NL) causes:	**Categories of changes and consequences**			
	Metabolic/endocrine	**Ovarian/endocrine**	**Functional/clinical**	**Breeding consequences**
Negative Energy Balance (NEB)	-Reduced synthesis and secretion of GnRH and LH -Reduced glucose -Reduced insulin -Reduced IGF-I	-Low or absent production of estrogens -Delayed LH-peak -Delay or absence of ovulation	-Poor estrous signs -Poor oocyte quality -Failure of fertilization -Early embryo death -Shorter estrous cycles -Anestrus	-Repeat breeding -Low pregnancy rates -Low calving rates -Extended calving interval -Economic losses -Poor animal welfare
	- Secondary mobilization of tissue reserves (with reduction of BCS) - Metabolic disorders (hypocalcaemia, acidosis, ketosis, fatty liver etc)	- Besides the above changes, also: - Increased levels of urea, beta-hydroxy butyrate, non-esterified fatty acids (NEFA) and triacylglycerol	- Impaired liver function - Impaired endometrial function (less favourable for embryo development) -Impaired immune function (increased propensity to endometritis and retained placenta)	
Increased metabolic hepatic rates	Increased catabolism of estradiol and progesterone	Lower levels of estradiol and progesterone in circulating blood	-Poor or absent estrous signs -Failure of fertilization -Early embryo death	

However, we need to remember that the cause of low fertility in dairy cows is multi-factorial, not only originated by lactational NEB. Poor nutritional management of the dairy cow, particularly before and after calving, is actually a key driver for infertility [10] owing to the intricate relationships between nutrition intake and hormonal pathways in dairy (***Figure 16***). As we have already mentioned, reproductive traits have low heritability (<5%), so a major part of the documented decline in reproductive performance can be attributed to commercially-driven changes in the dairy sector such as larger herds, housing changes and tying procedures, DIY-AI, use of TMR, etc, that make harder to properly manage the reproduction of the cows [12,19]. For instance, use of TMR during the dry period leads to a large variation in BC, which is later reflected in decreased fertility. A clear decrease in fertility can also be seen after DIY-AI (compared to trained AI-technicians), a matter of major concern for the need of more training, and advice, but also because some of these changes are causing problems of detection and (even worse) the intensity of display of estrous signs [12]. There is a strong global trend towards larger dairy herds and automatization of e.g. feeding and milking in most industrialized countries [64]. This trend is accompanied by shorter times for supervision and care of the individual animal, and an increased use of hired and often inexperienced short-term labor, often resulting in deteriorated reproductive management and fertility (***Table 2*** and ***Table 3***). Laborious estrus detection and poor conception rates have led to AI being replaced by natural services using cheap, untried bulls for returning cows, thus neglecting the advantages of AI and breeding selection. In North America and some European countries, mainly Scandinavia, the Baltic region, Austria and Switzerland, where a substantial part of the herds are still kept tied [65], there is a simultaneous trend towards loose housing in free-stalls, resulting from animal-welfare concerns and rationalization. The transition has a potential to facilitate behavioral expression of estrus and estrous detection. On the other hand, inferior barn design, limited space and low quality of flooring of alleys, passageways and holding areas have constrained the cow´s ability to display estrus [66] by affecting their locomotion and deteriorating claw health [67], further reducing reproductive performance (***Figure 14***).

A poor BC at calving has a significant negative impact on the probability of conception, the rate of embryonic loss, and the proportion of anestrous animals (33). Poor nutrition during the dry- and early postpartum periods results in reduced glucose, insulin, IGF-I and low LH pulse frequency with concomitant increases in beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA) and triacylglycerol [68]. Already at this stage, cows must mobilize large lipid reserves, but also some protein reserves, increasing the incidence of metabolic disorders like milk fever, acidosis, ketosis, fatty liver and displaced abomasum. Occurrence of milk fever and ketosis affect uterine contractions, delay calving and increase the risk of retained fetal membranes (RFM) and development of endometritis. Fat and/or overfed cows at calving (high BC) are those becoming problem cows when NEB is established [69] (***Figure 17***), implying that a high BC at calving is actually considered to be worse than a low BC, because high BC-cows eat less after parturition, have lower appetite, easily mobilize energy from body reserves, thus being more prone to suffer dramatic changes in BC. Moreover, a high BC appears to be one of the nutritional risk factors causing RFM, alongside with hypocalcemia and deficiencies in vitamin E and selenium [68]; metabolic disorders which, in turn, predispose cows to gynecological pathologies, of which endometritis postpartum is most important. Risk factors for endometritis postpartum are thus hypocalcemia, RFM, high triacylglycerol and NEFA levels (***Figure 18*** [70]). In sum, cows that are able to maintain the same BC (either low or high) manage better than those having fluctuating BC.

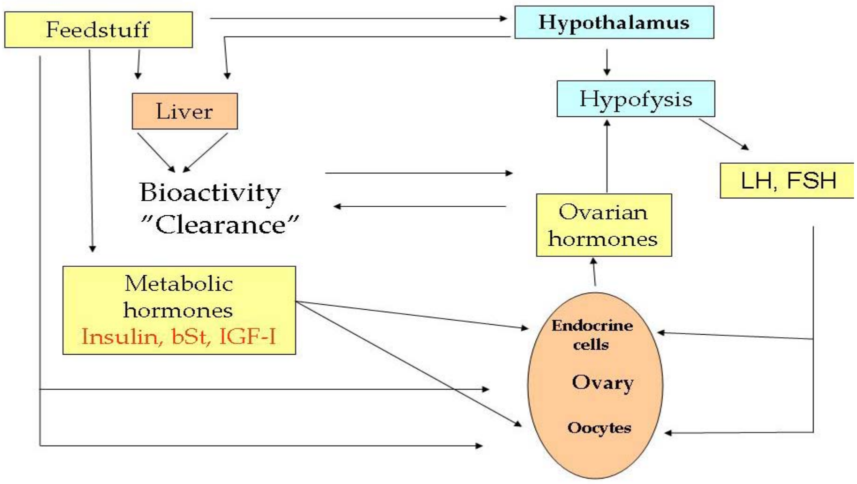

Figure 16: Relationships between nutritional intake and hormonal pathways in dairy cattle.

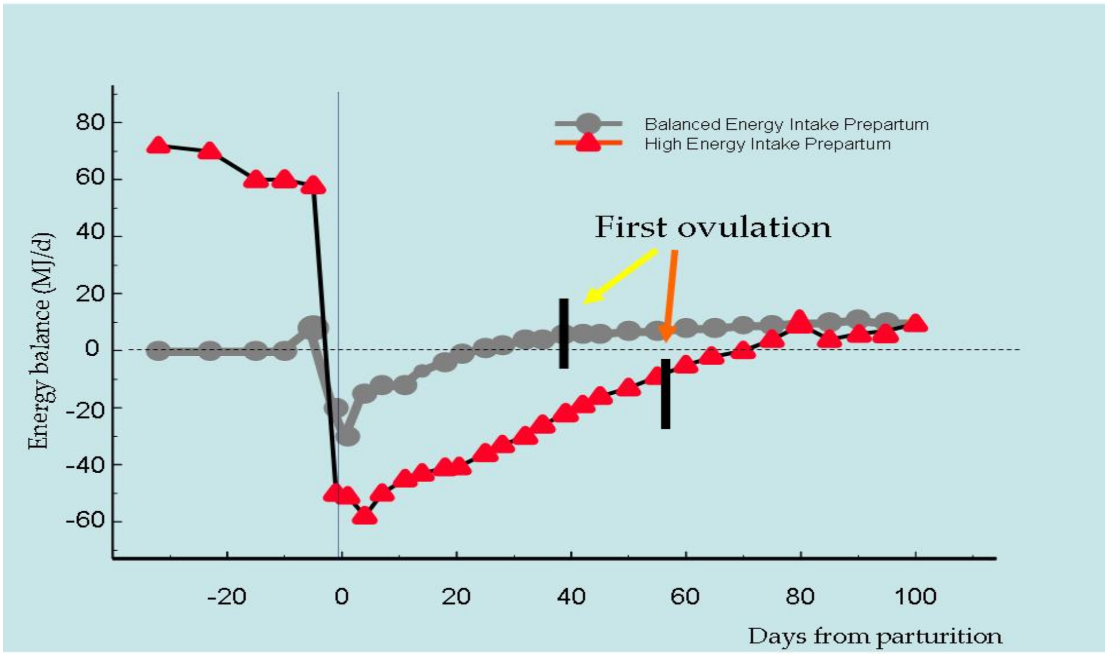

Figure 17: Overfeeding dairy cows pre-partum delays the occurrence of spontaneous first ovulation postpartum (Adapted from Rukkwamsuk et al, Vet Q 1999; 21).

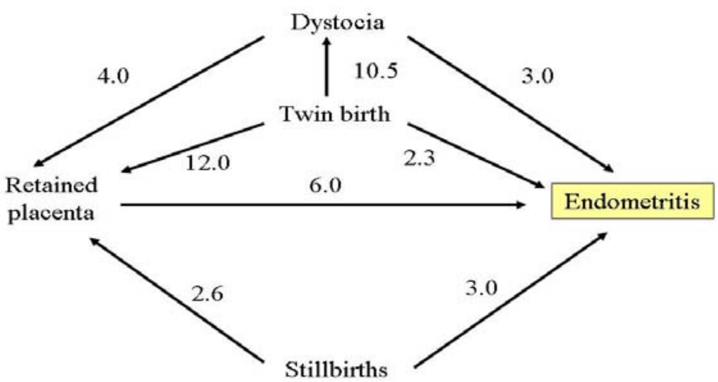

Figure 18: Risk factors (as Odd Ratios, OR) for endometritis in postpartum dairy cattle (Adapted from Smith & Risco, Comp Contin Educ Pract Vet 2002; 24).

Cows that are over-conditioned at calving [10] or that loose excess body weight (BW) are more likely to have a prolonged interval to 1st-estrus, thereby increasing the number of days open (DO). Recent evidence shows that dry matter intake (DMI) is the principal component of NEB influencing subsequent fertility [63]. Nutritionally-induced postpartum anestrus is characterized by a turnover of dominant follicles incapable of producing sufficient estradiol to induce ovulation due to a reduced LH pulse frequency. Lower concentrations of estradiol on the day of estrus are highly correlated with the occurrence of sub-estrus, thereby making the detection of estrus in high-yielding cows even more difficult. Nutrition also affects CR at following AI. Cows that develop hypocalcaemia, ketosis, acidosis or displaced abomasums have lower CRs and take longer time to become pregnant. Excessive loss of BC and excess protein content in the ration can reduce CR while supplementation with certain fats increase progesterone (P_4) concentration in blood plasma, attenuates the production of $PGF_{2\alpha}$, and can lead to an increased CR. The increased metabolic clearance rate of P_4, which decreases blood concentrations during early embryo cleavage up to the blastocyst stage is associated with decreased CR [71]. High nutrition levels can also increase the metabolic clearance rate of steroid hormones such as P_4 or estradiol [68] (see ***Figure 19***).

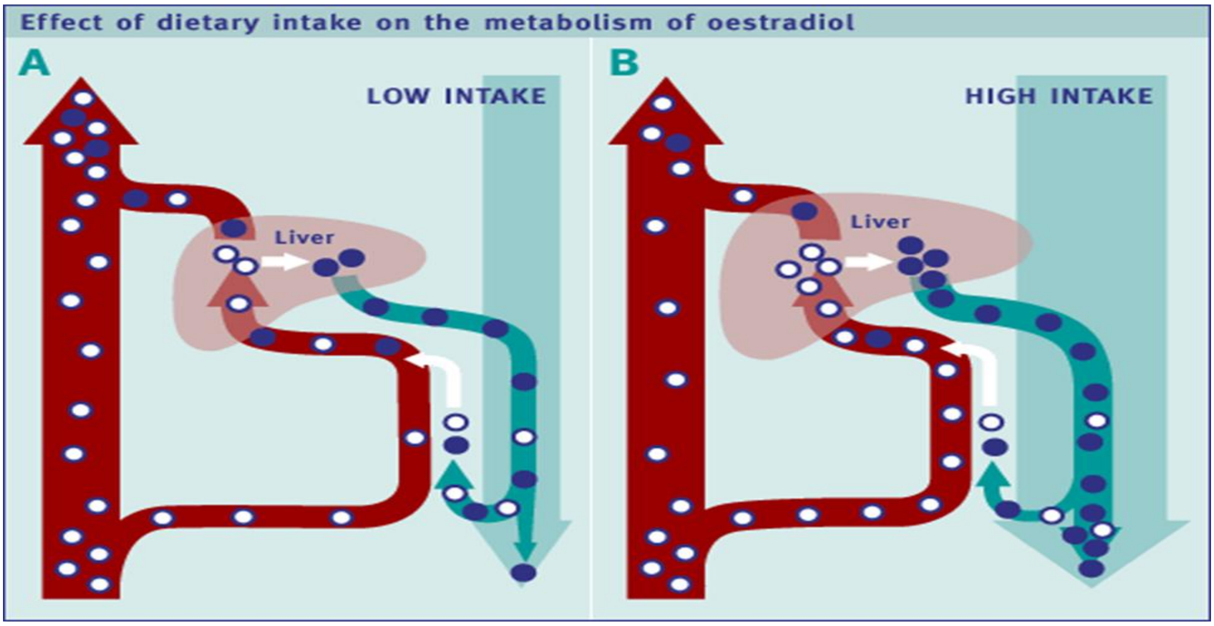

Figure 19: Effect of a high feed intake on the metabolism/clearance of estradiol (E_2). E_2, circulating in the blood stream, is normally inactivated in the liver by conjugation and excretion through the bile. In the intestine, bacteria re-activate the E_2 which is resorbed via the mesenteric blood stream and returned to the liver. At low feed intake (A), less E_2 is transported to the liver via the mesenteric blood pathway and thus the total E_2 metabolic rate is low. When feed intake increases (B) or it is high in large, high-producing dairy cows, the mesenteric blood flow increases and more E_2 reaches the liver, which increases in size to meet demands for the elimination of E_2, thus decreasing its levels in the animal with consequences for normal signalling during estrus (e.g. poorer estrus signs) (Source: Silvia WJ, 2003, http://www.vetsite.org/publish/articles/000043/article.html, with permission).

Genetic merit for high milk production often leads, over the year, to reduced reproductive performance, compared to cows with medium- or low-genetic merit, as determined for Holstein cows in southern USA [72](***Figure 20***). However, other studies have not detected associations between reproductive performance and milk production, feed intake or plasma concentrations of glucose, NEFA or IGF-I between calving and 1st-service [73], calling for caution when promoting these variables as predictors of reproductive performance.

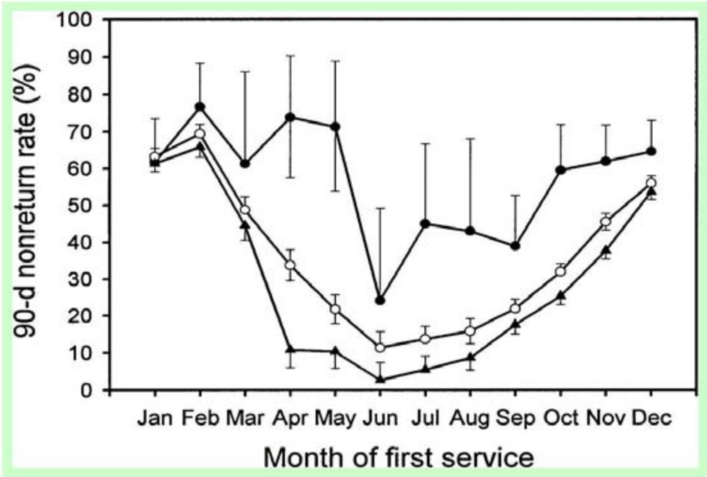

Figure 20**:** Seasonal variation in 90-d non-return rate (least-squares means ± SEM) to first service is affected by ECM milk yield (●<4536 kg; ○=4536-to-9072 kg; ▲>9072 kg) (Source: Al-Katanani et al, J Dairy Sci 1999; 82, with permission).

Specific causes of reproductive failure

Fertility in cattle has many pre-requisites and components, which require males and females to be functionally capable of reaching the last step, the birth of a normal, vital calf, thus defining the general breeding goal: cows should return to normal ciclicity early postpartum, show strong and regular estrous signs, conceive after AI, carry their pregnancy to term, calve easily and give birth to viable and healthy calves. Low calving rates relate to the inability of the cow to resume ovarian activity and thus failure to cycle and to express estrous signs (anovulatory and behavioral anestrus, irregular estrous cyclicity, etc); as well as to reproductive wastage due to fertilization failure, early and late embryonic mortality, fetal mortality (abortions) and stillbirths. Fertilization rates (% of ova being fertilized), are generally high after AI under controlled conditions. However, decreasing rates are now seen in high-producing Holstein cows [74]. There are probably many causes why 10–25% of ova are not fertilized after AI, and they can be of either male or female origin. But, to what extent does husbandry incide?

Sire fertility relates both to sperm numbers and their quality at the site of fertilization in the oviduct. The male not only contributes to fertilization in the female but also, through the quality of spermatozoa, to the ability of the embryo to survive throughout pregnancy [75]. However, many details concerning the structure and function of the spermatozoon are yet poorly understood. Spermaozoa are transcriptionally silent as a consequence of the highly condensed chromatin architecture and there is almost no cytoplasm capable of supporting translation. However, at least in primates and rodents, spermatozoa carry a full complement of mRNAs that can, under certain conditions, be translated *de novo* [76]. The spermatozoa delivered by fertile males contain up to 3,000 different mRNA species and regulatory micro-RNAs and non-coding RNAs. The functionality of these RNA molecules in fertilized embryos is largely unknown. Only some of these mRNAs have shown to be important through their established roles during development. Many of these mRNAs are male-specific, potentially affecting phenotypic traits in the offspring. This epigenetic phenomenon can involve the transmission of extra-chromosomal episomal elements. Recent studies have revealed that the presence of aberrant RNA in defective spermatozoa might influence and even disrupt early embryogenesis [77] but there is no information whether such aberrant RNA would cause epigenetic-like defects, visible later during fetal development [24]. Differences in individual transcriptome profiles in AI-bull sires have been recently shown, indicating a relation between presence of specific transcripts relevant for cell functions and the level of fertility after AI [78]. Determination of the sperm transcriptome is thus likely to increase our understanding of reproductive success (and failure), provided studies are done on proven AI-bulls, where all other possible confounding factors are minimized or, at least, controlled. Since the spermatozoon is a particular cell type, conventional microarrays do not presently contain all their transcripts, particularly those low abundant. Major attention must, therefore, be put on adaptations of current extractions protocols for spermatozoa.

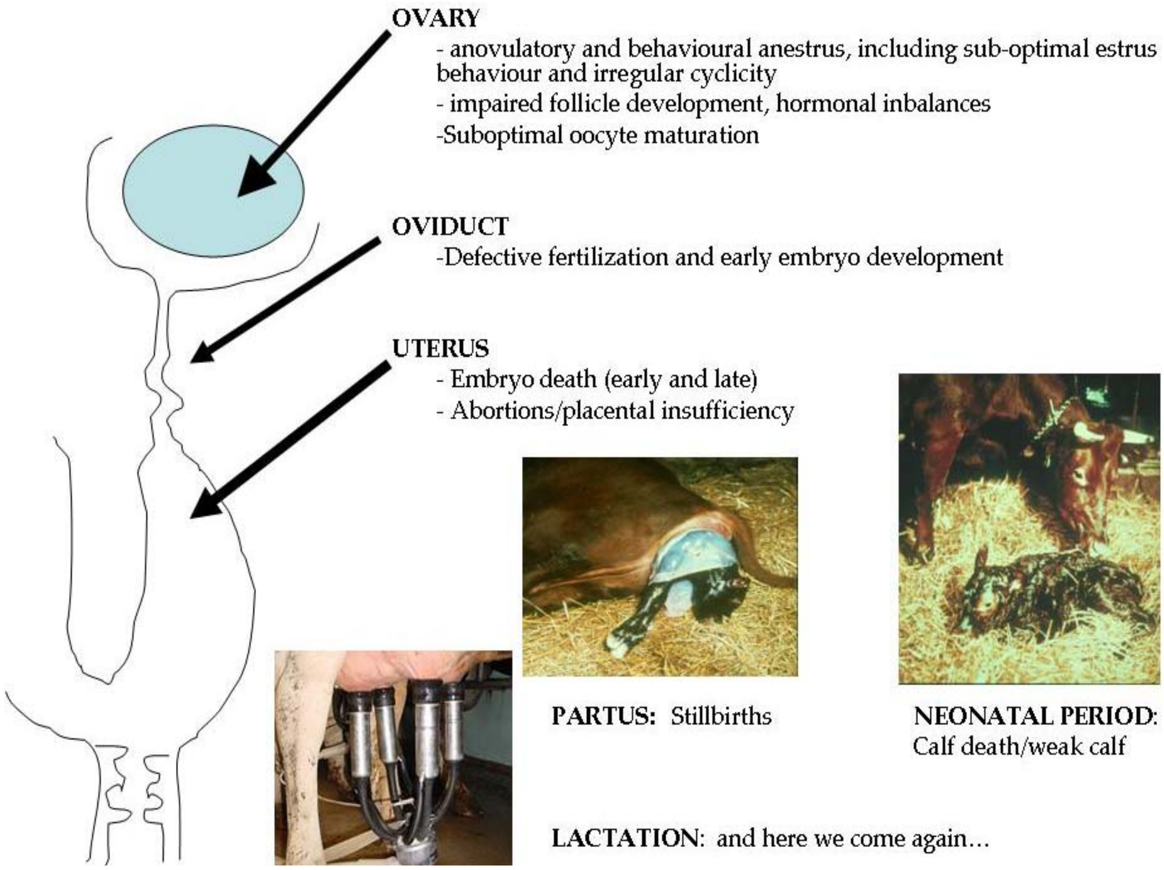

***Figure 21*:** Primary, non-infectious, causes for reproductive failure in high-producing dairy cattle.

Female fertility is influenced by numerous factors (see ***Table 4*** and ***Figure 21***). Establishment of lactation postpartum leads to the inhibition of LH pulsatility and a subsequent normal anovulatory anestrus (more dramatic when suckling is present). In suckling and low-milk yield cows, this period is followed by a resumption of gonadotrophic and ovarian activity and, most often, normal fertility. High-producing dairy cows, however, have often extended periods of anovulatory anestrus, as a consequence of the inadequate hormonal balance that the catabolic NEB causes (low blood levels of LH, insulin and IGF-I), resulting in impaired follicle development, estrous signs, LH surge and ovulation [79,80]. Moreover, high-producing cows in NEB experience a delayed onset of postpartum ovarian activity and reduced P_4 levels [81], the latter caused by a lack of ovarian luteinized tissue or a higher rate of metabolism of the hormone by increased feed intake [82]. High producing cows with these hormonal imbalances have shorter estrous cycles and depict fewer estrus signs than expected (***Table 1***, ***Figure 12***), owing to sub-optimal estradiol levels [83]. Hormonal imbalance (LH levels, metabolic rates), as well as genetic factors relate to the occurrence of twinning and cystic ovarian disease in high milk yield cows [84,85]. Oocyte quality, built upon a total maturation time in the ovary of around 3 months, is very sensitive to negative influences such as nutritional deficiencies or over-conditioning (***Table 4***). Accumulation of

NEFA derived from the adipose tissue during NEB in the follicle fluid constrains the proliferation and health of the granulosa cells and thus jeopardizes oocyte development [61,62]. Extension of the pre-ovulatory phase, *i.e.* delayed ovulation, due to suprabasal P_4-levels causes ageing processes in the oocyte and compromises fertility, leading to repeat breeding by fertilization failure [86]. After fertilization, an embryo is formed which develops in the oviduct during the first 3-4 days before it enters the uterus. The embryonic period lasts up to day 42 after fertilization and involves a series of critical periods, of which one comprises the first three weeks of development ("early embryonic death"), accounting for ~20% of the total losses. From this moment up to day 42, losses are termed "late embryonic death". While fertilization failure and early embryonic death relate to a low genetic index of the female, up to 25% of late embryonic deaths have been seen in cows with genetic potential for a high milk yield but mainly related to milk yield rather than to their genetic index [87]. Some of the early embryo losses might result from a malfunctional cytoplasm which impairs further development of the fertilized oocyte, a situation seen in over-conditioned repeat breeders [88]. Cytokines can adversely affect uterine function and indirectly cause early embryonic death in relation to mastitis during early lactation [89]. Early embryonic death can also result from a sub-optimal combination of genes arisen during fertilization. Genetic selection for milk production has also been related to early embryonic death owing to impaired oocyte quality [2], lowered P_4 concentrations [82], or both. As well, reduced levels of P_4 and of insulin-like growth factors can induce a sub-optimal uterine microenvironment, incapable of sustaining early embryonic life [90]. An early loss will lead to a new estrus (within 17–24 days from AI) giving the dam a new chance to conceive by minimal costs in time. However, there might be other reasons behind embryonic losses, even at a later stage, resulting in a costly delayed return to estrus. The early establishment of a pregnancy is controlled by complicated endocrine events, including the regulation of the expression of interferon-tau (IFTN), the maternal pregnancy recognition factor in the bovine. Owing to its role in regulating IFTN expression, the Fibroblast growth factor-2 (FGF2) gene relates to early embryonic death [91]. A hormonal asynchrony causes early embryonic death [86], and might be linked to the general trend of decreased fertility where lower levels and atypical temporal release (atypical profiles) of serum P_4 have been detected [92]. It is possible that high-producing cows have higher metabolic rates and a larger blood flow through the liver, which leads to lower concentrations of P_4 and oestradiol reaching the target cells [85]. Even husbandry-driven stress is nowadays regarded as a major contributor to endocrine disorders, causing repeat breeding and early embryonic death [93]. Excess rumen-degradable protein has been associated with increased embryonic mortality and, possibly, fertilization failure [94], because an excess of protein and a deficit of energy in the feed ration increases the production of ammonia that, when converted into urea in the liver, causes embryo mortality through an exacerbation of NEB and reduced plasma P_4-levels, an alteration of uterine pH and increased secretion of $PGF_{2\alpha}$ [95]. During the remaining gestation (day 43 to term, called fetal period), losses such as abortions are less prevalent, but an increased incidence (>12%) of early fetal loss (between 45 and 60 days of gestation [96] has been recorded and related to current intensive management systems [97], hot climate [98,99], and to animal factors of non-infectious nature [100,101,102], including twinning [96], and sire (often related to infections or carried genes). Plasma concentrations of pregnancy-associated glycoproteins (PAG) on day 35 of gestation can also indicate subsequent fetal loss with a strong relation to sire [97].

The epigenetic programming of the gamete genome is crucial for normal development and its modification influences heritable patterns of chromatin conformations and gene expression, leading to stable phenotypic differences among specialized cell types, as for instance those of the placenta [103]. Some syndromes in cattle such as the "large offspring syndrome" observed in *in vitro* produced animals or in cloned animals, have been considered the consequence of existing epigenetic errors in the donor cell genome or of an incomplete epigenetic reprograming during development [104,105]. Particular placentopathies in cloned founder cows reflect epigenetic effects [106], but there is yet much to unravel in order to define the molecular mechanisms involved and when they arise. For instance, whether aberrant paternal mRNA would cause epigenetic-like defects, visible either during early embryo development or later during fetal development, is yet to be determined [107], although seemingly related to the fertility of the males [78].

Calves born dead or dying within 24 hours are termed stillborn. Stillbirth is costly, not only because of the loss of the calf, but also because it leads to a higher risk for diseases in the dam and culling [108,109,110]. In Sweden, stillbirth rates are significantly higher in SH-heifers (first calvers, ~11 %) compared with SH cows and SR heifers or cows (6%), a difference that has increased during the last 20 years. Noteworthy, for second calvers, hardly any differences exist in either calving difficulty or stillbirth between breeds. Only half of the stillbirths are associated with calving difficulties indicating low fetal or calf vitality as one reason. Although it is a multi-factorial problem, the genetic factor is significant, as shown by an association between a higher proportion of Holstein genes and increased stillbirth rates, present already by the 1990´s [111]. Such a relationship was not present in Scandinavian Red breeds [21,112]. Being the 1st-parity animals overrepresented, this category should be the main source of information for further genetic evaluation.

Calf survival is also multi-factorial, including placental insufficiency, prolonged parturition, poor- or abnormal maternal behaviour, bad udder conformation, etc. Although the physiological cause behind the lower vitality of the fetus or calf is unknown, placental insufficiency is related to lower levels of placenta-specific hormones measured during the last six weeks of gestation in heifers with stillborn calves [113,114]. Whether such placental insufficiency relates to epigenesis, to hormonal imbalance under production or husbandry constraints, to maternal behaviour or the concerted interplay of these factors, remains to be determined. All these maternal factors can extend the interval from birth until 1st suckling, a simple indicator for low calf vitality [115]. Group-housing during calving might also cause delayed 1st suckling due to disturbing older or higher-ranked cows with a strong maternal interest in newborn calves [115].

Strategies to ensure good fertility in high-producing dairy cattle

As already mentioned, infertility in dairy cattle is multifaceted. Addressing it requires, therefore, a multi-disciplinary approach. However, not all solutions intended for amelioration of the problem are long-lasting, feasible or acceptable. Some measures can be applied rather immediately while others require further research and long-term strategies. From an EU perspective, discussions about animal ethics and welfare demand higher longevity of dairy cows and bans on the use of drugs for improving reproduction, especially hormones [16]. Emphasis is needed on the relation between management and genetic gains, considering that many of the current problems with dairy cow fertility are a logical consequence of the low profit margins of the dairy sector, which impinges for high milk yields. With a global trend of strict cost controls, increased herd sizes, and changing farming systems, there is an unfortunate association with shortages of skilled labour and less time to look for physiological signals in the herd, the basis for good management. Moreover, we should recognize our limited ability to prevent and treat diseases, to appropriately manage, feed and select dairy cows with desirable reproductive traits. Excellent papers on strategies for increasing fertility in dairy cows have been written [11,116]. Moreover, a summary of strategies proposed for restoring fertility in high-producing dairy cows is hereby presented in ***Table 5***.

***Table 5*: Strategies to ensure high fertility among high-producing dairy cattle: pros and cons.**

Term	Strategies		Pros	Cons
Short- & medium-term (ameliorative)	(i)	Manipulation of the estrous cycle and control of ovulation	Quick, variable results	Hormonal manipulation, welfare questionable
	(ii)	Dietary management	Attractive to combat NEB	Difficult to made routine, risky
	(iii)	Managing the dry period	Enhances DMI	Variable
	(iv)	Extended lactation and differential milking	Increases chances for conception	Decreases number of calves/cow, can lead to udder health problems (SCC), requires extra feeding.
	(v)	Use of high fertility bulls	Long-lasting, easy	Requires use of sires with fertility traits included in selection
	(vi)	Crossbreeding	Heterosis, combats inbreeding	Does not improve genetics
	(i)	Full-purpose buildings and management	Facilitates reproduction	Expensive, requires planning
Long-term (sustainable)	(i)	Correction for the weight of different functional traits in the breeding goal	Easy to apply	Can decrease milk yield figures
	(ii)	Prospective genetic selection strategies	Testing of young breeders-to-be, specific, lower costs	Emerging technologies, with uncertain applicability

Short- and medium-term strategies to ameliorate fertility problems

(i) Manipulation of the oestrous cycle and control of ovulation: From a veterinary medicine perspective, manipulation of the estrous cycle and the control of ovulation appears as a good short-term strategy. Application of methods to control the development of follicle growth, the promotion of ovulation in anestrus cows, the regression of the corpus luteum in cyclic cows and the synchronization of estrus and ovulation at the end of treatment, before AI (on spontaneous or expected estrus) or mating have been thoroughly studied [116,117]. However, most of these approaches imply the use of hormones (estradiol, P_4, GnRH, $PGF_{2\alpha}$) administered by different treatment regimes and with different intensities. Because there is large variation between countries regarding the availability and regulations of these treatments, it is difficult to consider this alternative as a feasible general strategy. A similar situation applies to the use of exogenous recombinant bovine somatotropin (rbST) around AI or of exogenous P_4 after ovulation, in order to increase expected low levels of IGF-I or P_4, respectively. Both hormones are banned already, or the public concern prevent their application. The need for multiple applications and the presence of a veterinarian (sometimes enforced by law) as well as the treatment costs, counteract their use. Moreover, we still do not know of eventual long-term effects of hormonal manipulations on genetics, thus shadowing our possibilities to safely select the best cows. Either way, these are no sustainable alternatives.

(ii) Dietary management: Use of diets designed to improve fertility by counteracting specific points in relation to NEB has always been an attractive way to circumvent the impairment of reproduction during early lactation that the partitioning of energy causes. However, the cow is biologically driven to mobilize body fat when she is fatter than her biological target condition [118], which makes this strategy difficult. Several ways have been attempted to reduce the effect of NEB, namely (a) the reduction of BC at calving, to avoid the limiting negative feedback effect of body fat on the cow´s DMI (the procedure require high-quality diets are available for these -hopefully- "thinner" cows [119]); (b) the feeding of low-protein diets that reduce body-fat mobilization by provoked imbalance in the protein-to-energy ratio [120]; (c) the change of carbohydrate source in the diet to increase dietary energy concentration (increasing starch or fat and decreasing forage content, a risky procedure because of its implications on rumen function, milk composition, nutrient partition and metabolic hormones [121]; and (d) the inhibition of milk fat synthesis with exogenous conjugated linoleic acid (trans-10, cis-12 CLA), thus limiting energy output in milk [122,123].

Moreover, although there are specific nutrients designed to trigger the endocrine system of the cow during early lactation; one must consider that while insulin (as well as GH, IGF, LH and leptin) can profoundly influence ovarian function (follicular development in particular [124]), the roles of GH, IGF-I and leptin appear to be more related to milk yield, BC and LW than with the whole nutritional status. Changes in diet composition can, on the other hand, elicit large changes in insulin levels, since plasma insulin concentrations are positively related to dietary starch concentration. Therefore, attempts have been made to use exogenous propylene glycol [125] or hyperinsulinemic diets [126] with the purpose of "fooling" the cow into a virtual anabolic condition, increasing glucose and insulin concentrations in circulating blood. However, insulin (directly or via glycogenic substance) has differential effects, being able to stimulate resumption of cyclicity [127] but also cause detrimental effects on oocyte competence [128]. Moreover, it has recently been shown that insulin alters the enzymes responsible for the catabolism of P_4 in the liver, thus decreasing the decay of the hormone [129]. A recent review on the relation between nutrition of high-yield dairy cows and oocyte and embryo quality has been published [130].

On the other hand, exogenous conjugated linoleic acid (CLA) seems to excert positive effects on oocytes, and to increase P_4 and $PGF_{2\alpha}$ levels [131]. Combinations of these designed diets are obviously more attractive, because they have proven to increase pregnancy rates dramatically (from 27 to 60%), implying that cows producing ~10,000 kg would have a fertility comparable to cows producing ~6,000 kg, by increasing insulin status immediately postpartum, and then reducing insulin status during the mating period [132].

(iii) Managing the dry period: Shortening or eliminating the dry period has been postulated as a suitable way to quickly enchance fertility in dairy cows. This management can increase DMI during the transition period, decrease milk energy output, or both. By increasing the energy status of dairy cows, there is an indirect increase in reproductive efficiency [40]. However, such practice does not apply in general as it may have negative effects on udder health and total milk yield, and should be considered on a herd-to-herd basis.

(iv) Extended lactation and differential milking: Most veterinary attention in dairy cattle is required from one week before to 10 weeks after calving [133], confirming that calving is a welfare risk. Moreover, it is important to avoid the impact of NEB on the resumption of reproductive function. For these reasons, voluntarily delaying the 1st postpartum AI, and attempting to have the cow calving at a calving interval of maybe 18 months or so, leads to (a) the cow prolonging its lactation (so-called "persistent" lactation), (b) no need to look for estrus during the milk peak at early lactation, and (c) with AI done later during lactation, the cow having a better chance of getting pregnant [134,135]. On the other hand, because different cows have individual lactation curves, it seems difficult to select those individuals which are to be grouped for short respectively long calving intervals. Increasing milking frequency [136] by the use voluntary milking (using robotics) promotes extended lactation, but leads to a delay in the appearance of ovulatory estrus. Moreover, using extended calving intervals means fewer calves are born per cow, persistent lactation can lead to udder health problems in cows with high somatic counts and milk production can only be reasonably maintained by compensatory feeding during the declining phase. To be economically acceptable for the farmer, the milk yield has to be maintained over time [46] and thus requires proper management [137]. An alternative strategy is to flatten the peak of the milk curve by employing once-daily milking in early lactation (but risking udder problems…). Such practice promotes earlier resumption of ovarian cyclicity by increasing nutritional status, avoiding the impact of NEB and, further, the cow maintaining BC throughout lactation [138]. It may also be an alternative to genetically select for flatter lactation curves.

(v) <u>Use of high-fertility bulls</u>: Use of AI with semen from sires with proven high-fertility is probably the most obvious and simple recommendation. However, in order to prolong the term of this strategy from short to medium, the breeding selection must be appropriate, *i.e.* including fertility traits with a certain weight in order to warrant that the improvement of reproductive performance is selected while still maintaining enough yearly increase in milk yield, thus warranting the rentability of the dairy production in a longer perspective.

(vi) <u>Crossbreeding</u>: Use of semen from other breeds where the decline in fertility is not a severe problem is also a medium-range alternative to halt fertility deterioration, although it might not be the best long-term strategy. The procedure is already customary in New Zealand where Jersey is crossed with Holstein [139], and has proven attractive for other markets [140], provided the semen comes from appropriately progeny-tested sires, and with production levels close to the breed in question. A valid example is the use of SR semen for crossbreeding on Holstein, now attaining production levels quite similar to those pure Holsteins, but with superior fertility [139,141]. This strategy can also be used to combat inbreeding in herds where the problem is large. In particular, the development of multiple lines with similar capacity for milk production is attractive, based on the assumption that crossbreeding could be used to capitalize on heterosis [116]. However, we should always bear in mind that cross-breeding is not per se genetic improvement and that genetic selection is still needed within the breeds used.

(vii) <u>Full-purpose buildings, management and automated systems</u>: Well-designed cow barns, with good width and slip resistance of the flooring of alleys, passageways and holding areas suport best animal well-being and allow for efficient health management, including reproduction, by promoting expression of behavioural estrus, and providing the best opportunities for estrus detection by the staff [66]. However, detection of estrus or of health problems (lameness, for instance) is often constrained by the shortage of skilled and experienced personnel to spend enough time with the animals. This problem is, unfortunately, aggravated by the increasing display of weaker signs of oestrus by high-producing cows, which are not always even detected by the use of mounting-detectors [12]. For these reasons, automated systems or the supervision have been designed, including those monitoring the activity by the cows (as ALPRO™ [www.delaval.com)] or Afiact™ [www.afimilk.com], among others available in the market) with heritabilities of ~0.17 for estrus control [142]. Other methods relate to specific hormones. For instance, P_4 concentrations in blood or milk can be used to monitor the interval from calving to initiation of luteal activity, with a heritability of ~0.2 [143,144,145,146]. Owing to the close relationship between P_4 levels, peak milk production and ML of the dairy cow, P_4 profiles appear suitable to evaluate fertility values of individual cows [92,145] even at infrequent intervals (e.g., once monthly [146]). Different fertility variables can then be derived from this P_4-profile, as the interval from calving to initiation of luteal activity, the interval from calving-to-ovulatory-oestrus, etc, variables that are less affected by management decisions and more influenced by the cows' own physiology. Perhaps more importantly, P_4 levels in milk can today be determined on-line, together with other indicators for presence of preclinical mastitis (lactate dehydrogenase LDH), hepatic function (beta-hydroxybutyrate, BHB) and of intake protein balance (urea), using automated equipments (such as Herd-navigator, www.herdnavigator.com). This on-line detection allows the diagnosis of problems and help improving the management of the high-producing cow at an individual basis. However, these indicators can only aid solving the individual problems that appear. Unless they are used to perform proper selection of the animals, the equipment will simply be another diagnostic tool (albeit economical for large exploitations). However, if linked to activity recorders, they may improve our capability to better time estrus and thus lead to a better timing of AI. Other useable markers are easier, such as the BCS, but there are no automated systems yet available for this indicator.

Long-term strategies to reach a sustainable improvement of dairy cow fertility

<u>(i) Correction for the weight of different functional traits in the breeding goal</u>: Following the Scandinavian decision to include fertility traits in the breeding goal for selection of dairy cattle (31), many countries are now incorporating similar approaches in their selection strategies [7], but the relative weight of health and reproduction still varies largely as indices of breeding selection (e.g., from 0.03 to >30% [25]). As already mentioned, reproductive traits have low heritabilities, but their genetic variation is large, thus making it possible to genetically select for good fertility without constraining the gains in milk yield [11]. Thus, a higher weight for *breeding values of several functional traits such as fertility, claw health, longevity and milk yield* have to be applied. Over many years, this has been proven in Scandinavia, where adjustments of the weight of different functional traits can provide more appropriate breeding indices for selection [17] and even increase farmer´s profits [147]. These data suggest that the current situation with dairy reproduction genetics is not a *cul-de-sac*. We need simply to work towards a lower progress regarding milk yield but with major gains in cow health. Economic calculations can be done for each of the weightings so that farmers and breeding organizations can best argument for the value of the changes towards sustainability, thus avoiding unnecessary, short-sighted discussions [148]. Moreover, we should perhaps re-consider the use of the trait measurements we have used for more than 50 years! The genetic accounting systems (as Best Linear Unbiased Prediction, BLUP; Animal Model, AM; Test Day Model, TDM etc) might not fully compensate for the recent dramatic changes we have experienced in environmental requirements and management practices. Perhaps we should consider the replacement of “non-return rate (NRR)” by “pregnancy rate (PR)” when basically all high-producing cows are pregnancy-tested using various means (trans-rectal palpation, ultrasound, pregnancy-specific protein determinations, etc). Such considerations seem imperative in the face or our inability to accurately detect estrous signs.

We should also strive to develop accurate and practical methods and determine best markers for the complex physiological traits that relate to fertility constraints, to measure welfare traits under different environmental conditions and incorporate them into the breeding goals and selection schemes [21]. As an example, we need to determine the strength of display of estrus signs, when conceptus losses occur in relation to characterstics such as breed, milk yield, production-related pathologies or husbandry. Without these markers, our capacity to design proper strategies is undermined. Markers must also be practical in order to ensure commercial use. Use of on-line measured indicators (see above <u>point *vii*</u>) can, provided that the monitoring can be done at low cost and the system widely adapted for most on-farm use, lead to information be easily incorporated into the selection schemes for bull sires [149]. In any case, the most important trait measurements should be based on classical physiological data such as P_4 levels, estrous activity, BCS, but also on novel ones such as measurements of the strength of estrous signalling (rather than the classical standing or mounting marks), or the steroid clearance in the liver (with cows selected for low steroid hepatic clearance rate). In this respect, we also should consider to strengthen the selection weight for the timing of the 1st ovulation postpartum, the earlier the better, as it is performed in other species. Indicators for this trait are available, such as P_4 levels (see above). To optimize fertility we also need to consider the quality of the bull semen with regards to RNA content as described above.

As mentioned already, another potentially useable marker is the BCS, a moderately heritable (0.09-0.45) trait favourably related to fertility and survival [108]. Selection for milk yield increases the NEB and lowers the BC of high-producing dairy cows, particularly when these animals have also been selected for "angularity" (dairy phenotype, see ***Figure 11***), a trait that has a strong genetic relation with BC [150]. Because there are positive genetic associations between BC and reproductive performance [151], selection programs based on mid-lactation BCS (when the genetic variance for BCS is largest and the genetic correlations between BCS and fertility strongest [152]). The BC is being used as a predictor trait for genetic merit for fertility in The Netherlands [153] as well as in Ireland and the UK [108]. Increasing the BC of dairy cows by selection would perhaps increase their genetically-determined set point for BC during lactation and thus diminish the effects of NEB, if present [154]. Such thoughts have lead to the "re-development" of other concepts regarding the need for appropriate selection of dairy cows, such as the concept of developmental programming, which basically implies that the plane of maternal nutrition can have an impact on the reproductive function of the descendant and its fertility [155]. To this end, our current use of traditional breeding value estimation procedures, and ot multitrait selection based on large databases and biobanks, might need from the inclusion of other, more suitable markers, to provide further gain in genetic evaluations, where traits such as milk yield, calving interval and survival can be combined to provide selection for longevity while maintaining acceptable levels of milk production [11]. However, these methods are still too costly and too slow to resolve the problems we face today.

<u>(ii) Prospective genetic selection strategies</u>: Over the past decade, whole-genome scanning using microsatellite markers on specific experimental designs, has led to the identification of several quantitative trait loci (QTL) for characteristics related to health, fertility and production, including single trait QTLs for the maternal (chromosomes 18, X/Y) and paternal (chromosomes 10, 18) effect on NRR [113]. Use of QTL in selection is most beneficial for low heritability traits, sex-linked traits and traits expressed late in life, such as daughter fertility, a commonly used breeding value in selection (in Sweden, since he early 1970s [21]). Multiple trait QTL regions have also been found for the combination of non-return rate and udder characteristics, and between production traits and non-return rate [156]. Linkage to genetic markers have also included ovulation rate and multiple ovulations, associated with QTL on chromosome 7 [157], a chromosome where many genes linked to endocrine and fertility aspects of dairy cows have been identified. However, because the region of interest as derived from a QTL scan is often quite large and can contain several hundred genes, there is a need for the simultaneous profiling of gene expression of many genes by RNA expression arrays. One of the available strategies is the candidate gene approach, where physiological findings are used to identify genes whose variation can have influence the trait of interest. The genetic variation between animals in a particular gene (such as that coding for GnRH [158] or the bLH receptor [159]) is, at the end, linked to the phenotypic information.

However, we must bear in mind that fertility is one of the most difficult and complex traits owing to low heritabilities caused by the polygenic nature of reproductive traits and the strong environmental influences on reproduction and the long generation intervals in the bovine. In fact, thus far, no gene with a causative mutation has been identified, which underlies a detected QTL effect concerning reproductive traits in cows. For bovine fertility, many genes account only for a small amount of the phenotypic variation, the rest being caused by the environment. This is probably why, despite several identified QTLs have been incorporated into selection strategies by breeding companies globally, the rate of success is low, or very low (rev by [21]). Moreover, the identification of the variation in the genes involved is very costly, mostly because it is time-consuming.

The DNA microarray technology, which includes various versions of bovine microarrays, provides a tool for the analysis of tens of thousands of genes simultaneously, so that transcriptional and genomic changes can be identified on a global scale. To date, public databases (such as http://www.ncbi.nlm.nih.gov/genome/guide/cow/) comprise expressed genes from a multitude of bovine tissues, including embryos. Gene expression profiling using this technology for functional genomics is expected to aid the identification of genes and/or gene networks, that can be best linked to fertility and its evident polygenic trait character, thus allowing for genomic selection. Studies on epigenetics, using high-throughput methods for analyses of DNA cytosine methylation patterns are likely to provide support for specific areas relevant for fertility, such as early embryonic development and placenta formation and function, where maternal and paternal genome components interact [160].

Most likely, advances in the recording of Single Nucleotide Polymorphisms (SNPs) are being made, particularly with regard to the speed of genotyping individual animals for many tens of thousands of SNPs, thus opening for the inclusion of genome-wide marker information in the prediction of breeding values. Genomic selection of young bulls is ongoing in The Netherlands [161], Norway and Denmark/Sweden (cited by [21]). Genome-wide association analysis of tissue samples using whole-genome SNP-arrays (such as the BovineSNP50 BeadChip, Illumina Inc, USA [www.Illumina.com]) would provide information regarding the most important genetic factors that influence fertility and reproduction. Such genomic selection predicts breeding values for a large number of those haplotypes across the entire genome that are derived from combinations of marker alleles, thus increasing the degree of security for breeding values by 20-30%, compared to today´s methodology [162,163]. Selection programs based on such information should improve reproductive success in the future, considering more information shall be gathered for attributes with low hereditability such as fertility [162]. The most obvious application for this yet developing technology focus on dairy calves (which obviously do not have any phenotypic records yet), so that conventional progeny testing can, ultimately, be virtually waived, with enormous cost savings [164]. However, although these emerging technologies are yet to be fully established and cross-checked with the evolving traditional breeding estimations, so that the risk of late detection of undesired side-effects of selection are minimized, they promise to have a large impact on our understanding about the genetic factors controlling reproductive success.

Concluding remarks

The current situation with dairy reproduction genetics is not a *cul-de-sac*. However, we need to work towards a more balanced progress regarding milk yield and cow health, by either a direct selection for strategic fertility traits or by indirectly selecting for longevity or body condition score. The success shown by the Nordic countries in keeping a largely unchanged genetic trend in female fertility and calving traits while increasing genetic gains in production has shown this is possible. Either way, it requires using appropriate weightings of the available breeding values in traditional breeding analyses systems, or a combination of these traditional systems with novel developments in genome-mapping and functional genomics, pertaining genomic selection. However, because this long-term, hopefully permanent solution will take time to become effective, in the mid-term, we need to gain a greater understanding of the interactions between nutrition, management and fertility to better manage the current negative trends in dairy cattle health and welfare. More awareness of barn constructions to help appropriate display and detection of estrous signs, as well as better flooring, more and safer (less stressful) space for animal-animal and animal-man interaction are pending solutions. Application of specially designed diets, modification of body condition and milking profiles, and better use of markers (behaviour, P_4-profiles, estrus selection tools, etc.) are mandatory. Increasing our yet fragmentary knowledge on reproductive losses before calving is essential both in terms of the period of occurrence (embryonic respectively fetal), and the relationship between sire influence (potential epigenetic effects), embryo potential, and placental health, the latter determining the presence of stillbirth or the birth of weak calves. Last but not least, use of appropriate sires for breeding remains the best option for genetic improvement of fertility while yet maintaining high levels of milk yield.

It is clearly imperative for the breeding and dairy industry to put more emphasis on fertility, health and longevity, as well as for the pertinent research organisations and SME´s to engage in trait-orientated research, in order to be able to measure traits properly and at low cost, and to ensure that they are incorporated into the selection schemes, alongside with a possibly increasing use of genomic information. However, unless we gather more information on accurate phenotypes, there is an inherent risk that the molecular approach, such as genomic selection, lacks proper counter-information on animal physiology, behaviour and pathological constraints, thus becoming less beneficial than expected.

Acknowledgements

The authors´ own studies have been supported by grants from FORMAS and the Swedish Farmers´ Foundation for Agricultural Research (SLF), Stockholm, Sweden.

References

1. Sørensen AC, Lawlor T, Ruiz F. A survey on fertility in the Holstein populations of the world. Proc. Int Conf on Fertility in dairy cows, Liverpool Hope University, UK, 30-31 August 2007 (EAAP Satellite Meeting), 2007; 1: 17.
2. Humblot P. Use of pregnancy specific proteins and progesterone assays to monitor pregnancy and determine the timing, frequencies and sources of embryonic mortality in ruminants. Theriogenology 2001; 56: 1417-1433.
3. Andersen-Ranberg IM, Klemetsdal G, Heringstad B, Steine T. Heritabilities, genetic correlations and genetic change for female fertility and protein yield in Norwegian dairy cattle. J Dairy Sci 2005; 88: 348-355.
4. Royal MD, Darwash AO, Flint APF, Webb R, Wooliams JA, Lamming GE. Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. Anim Sci 2000; 70: 487-501.
5. Sartori R, Gümen JN, Guenther JN, Souza AH, Caraviello DZ, Wiltbank MC. Comparison of artificial insemination versus embryo transfer in lactating dairy cows. Theriogenology 2006; 65: 1311-1321.
6. Bousquet, D, Bouchard E, DuTremblay D. Decreasing fertility in dairy cows: Myth or reality? Proc 23rd World Buiatrics Congress, Quebec, Canada, July 11-16, 2004, 6 pp (http://www.ivis.org/proceedings/wbc/wbc2004/WBC2004-Bousquet-ang-simple1.pdf).
7. Lucy MC. Non-lactational traits of importance in dairy cows and applications for emerging biotechnologies. New Zealand Vet J 2005; 53: 406-415.
8. Kuhn M, Hutchinson J. Factors affecting heifer fertility in US Holsteins. J Dairy Sci 2005; S88: A11.

9. Pryce JE, Royal MD, Garnsworthy PC, Mao IL. Fertility in the high-producing dairy cow. Livest Prod Sci 2004; 86: 125-135.
10. Beever DE. The impact of controlled nutrition during the dry period on dairy cow health, fertility and performance. Anim Reprod Sci 2006; 96: 212-226.
11. Veerkamp RF, Beerda B. Genetics and genomics to improve fertility in high producing dairy cows. Theriogenology 2007; 68S: S266-S273.
12. Löf E, Gustafsson H, Emanuelson U. Associations between herd characteristics and reproductive efficiency in dairy herds. J Dairy Sci 2007; 90: 4897-4907.
13. Roxström A, Strandberg E, Berglund B, Emanuelson U, Philipsson J. Genetic and environmental correlations among female fertility traits and milk production in different parities of Swedish Red and White dairy cattle. Acta Agric Scand, Sect A, Anim Sci 2001; 51: 7-14.
14. Roxström A, Strandberg E, Berglund B, Emanuelson U, Philipsson J. Genetic and environmental correlations among female fertility traits and the ability to show oestrus, and milk production. Acta Agric Scand, Sect A, Anim Sci 2001; 51: 192-199.
15. Hultgren J, Manske T, Bergsten C. Associations of sole ulcer at claw trimming with reproductive performance, udder health, milk yield, and culling in Swedish dairy cattle. Prev Vet Med 2004; 62: 233-251.
16. Oltenacu PA, Algers B (2005) Selection for increased production and the welfare of dairy cows: Are new breeding goals needed? Ambio 34: 311-315.
17. Lindhé B. (Where is our breeding work going?] Vart är vi på väg i avelsarbetet? (In Swedish), Avelskuriren 2007; 2: 3-4.
18. Chagas LM, Bass JJ, Blache D, Burke CR, Kay JK, Lindsay DR, Lucy MC, Martin GB, Meier S, Rhodes FM, Roche JR, Thatcher WW, Webb R. Invited review: New perspectives on the roles of nutrition and metabolic priorities in the subfertility of high-producing dairy cows. J Dairy Sci 2007; 90:4022-4032.
19. Lucy MC. Reproductive loss in high-producing dairy cattle: Where will it end? J Dairy Sci 2001; 84: 1277-1293.
20. Funk DA. Major advances in globalization and consolidation of the artificial insemination industry. J Dairy Sci 2006; 89: 1362-1368.
21. Berglund B. Genetic improvement of dairy cow reproductive performance. Reprod domest Anim 2008; 43 S2: 89-95.
22. Christie MF, Allcock J, Royal MD. Reproductive challenges facings today´s dairy industry. Proc. Int Conf on Fertility in dairy cows, Liverpool Hope University, UK, 30-31 August 2007 (EAAP Satellite Meeting), 2007; 1: 1.
23. Maas JA, Garnsworthy PC, Flint AP. Modelling responses to nutritional, endocrine and genetic strategies to increase fertility in the UK dairy herd. Vet J (in press).
24. Miller D. Spermatozoal RNA as reservoir, marker and carrier of epigenetic information: implications for cloning. Reprod Domest Anim 2007; 42 (Suppl 2): S2-S9.
25. Miglior F, Muir BL, Van Doormaal BJ. Selection indices in Holstein cattle of various countries. J Dairy Sci 2005; 88: 1255-1263.
26. Hansen LB. Consequences of selection for milk yield from a geneticist´s viewpoint. J Dairy Sci 2000; 83: 1145-1150.
27. Gutierrez CG, Gong JG, Bramley TA, Webb R. Selection on predicted breeding value for milk production delays ovulation independently of changes in follicular development, milk production and body weight. Anim Reprod Sci 2006; 95: 193-205.
28. Carlén E, Strandberg E, Roth A. Genetic parameters for clinical mastitis, somatic cell count, and milk production traits in the first three lactations of Swedish Holstein cows. J Dairy Sci 2004; 87: 3062-3080.
29. Svensson C, Nyman AK, Persson Waller K, Emanuelson U. Effects of housing, management, and health of dairy heifers on first lactation udder health in southwest Sweden. J Dairy Sci 2006; 89: 1990-1999.
30. Emanuelson U, Danell B, Philipsson J (1988) Genetic parameters for clinical mastitis, somatic cell counts, and milk production estimated by multiple-trait restricted maximum likelihood. J Dairy Sci 71: 467-476.
31. Philipsson J, Lindhe B. Experiences of including reproduction and health traits in Scandinavian

dairy cattle breeding programmes. Livest Prod Sci 2003; 83: 99-112.
32. Veerkamp RF, Beerda B, van der Lende T. Effects of genetic selection for milk yie1d on energy balance, levels of hormones, and metabolites in lactating cattle, and possible links to reduced fertility. Livest Prod Sci 2003; 83: 257-275.
33. Weigel KA. Prospects for improving reproductive performance through genetic selection. Anim Reprod Sci 2006; 96: 323-330.
34. Windig JJ, Calus MPL, Veerkamp RF. Influence of herd environment on health and fertility and their relationship with milk production. J Dairy Sci 2005; 88: 335-347.
35. Windig JJ, Calus MPL, Beerda B, Veerkamp RF. Genetic correlations between milk production and health and fertility depending on herd environment. J Dairy Sci 2006; 89: 1765-1775.
36. Wade GN, Jones JE. ''Neuroendocrinology of nutritional infertility'', American Journal of Physiology, Regulatory Integrative Comparative Physiology, 2004; 287: 1277-1296.
37. Agnew RE, Yan T, Murphy JJ, Fems CP, Gordon FJ (2003) Development of maintenance requirement and energetic efficiency for lactation from production data of dairy cows. Livest Prod Sci 82: 151-162.
38. Knight CH, Beever DE, Sorensen A. Metabolic loads to be expected from different genotypes under different systems. Metabolic stress in dairy cows. British Soc Anim Sci 1999; Occas Publ 24: 27-36.
39. Veerkamp RF. Se1ection for economic efficiency of dairy cows using information on liver weight and feed intake: A review. J Dairy Sci 1998; 81: 1109-1119.
40. Grummer RR. Strategies to improve fertility of high yielding dairy farms: Management of the dry period. Theriogenology 2007; S68: S281-S288.
41. Ingvartsen KL, Dewhurst RJ, Friggens NC. On the relationship between lactational performance and health: is it yield or metabolic imbalance that causes diseases in dairy cattle? A position paper. Livest Prod Sci 2003; 83: 277-308.
42. Hjertén J. [Relationships between body condition score, subcutaneous fat, live weight and reproduction in Swedish Holstein and Swedish Red and White Cattle]. In Swedish. Summary in English. MSc thesis 281, SLU, Dept of Animal Breeding and Genetics, Uppsala, Sweden 2006; 35 pp.
43. Collard BL, Boettcher PJ, Dekkers JCM, Peticlerc D, Schaeffer LR. Relationships between energy balance and health traits of dairy cattle in early lactation. J Dairy Sci 2000; 83: 2683-2690.
44. Jensen MB, Munksgaard L, Pedersen LJ, Ladewig J, Matthews L. Operant conditioning as a method to assess lying motivation in dairy heifers. Proc 36th Int ISAE Congr, The Netherlands August 6-10 2002, p. 69.
45. Lidfors L, Isberg L. Intersucking in dairy cattle- review and questionnaire. Appl Anim Behav Sci 2002; 42: 87-94.
46. Dobson H, Smith RF, Royal MD, Knight CH, Sheldon IM. The high-producin dairy cow and its reproductiv eperformance. Reprod Domest Anim 2007; 42 (Suppl 2): 17-23.
47. Rajala-Schultz PJ, Gröhn YT. Culling of dairy cows. Part I. Effects of diseases on culling in Finnish Ayrshire cows. Prev Vet Med 1999a; 41: 195–208.
48. Rajala-Schultz PJ, Gröhn YT. Culling of dairy cows. Part II. Effects of diseases and reproductive performance on culling in Finnish Ayrshire cows. Prev Vet Med 1999b; 41, 279–294.
49. Rajala-Schultz PJ, Gröhn YT. Culling of dairy cows. Part III. Effects of diseases, pregnancy status and milk yield on culling in Finnish Ayrshire cows. Prev Vet Med 1999c; 41, 295–309.
50. Onyiro OM, Brotherstone S. Genetic analysis of locomotion and associated conformation traits of Holstein-Friesian dairy cows managed in different housing systems. J Dairy Sci 2008; in press.
51. Manske T. Hoof lesions and lameness in Swedish dairy cattle; prevalence, risk factors, effects of claw trimming and consequences for productivity. PhD-Thesis, SLU, Skara, 2002.
52. Søgstad AM, Østeras O, Fjeldaas T. Bovine claw and limb disorders related to reproductive performance and production diseases. J Dairy Sci 2006; 89: 2519-2528.
53. Telezhenko E, Bergsten C. Influence of floor type of the locomotion of dairy cows. Appl Anim Behav Sci 2005; 93: 183-197.

54. Cook N, Nordlund K. Behavioural needs of the transition cow and considerations for special needs facility design. Vet Clin North Am, Food Anim Pract 2004; 20: 495-520.
55. Von Keyserlingk MA, Olenick D, Weary DM. Acute behavioural effects of regrouping dairy cows. J Dairy Sci 2008; 91: 1011-1116.
56. Beilharz RG. Quantitative genetics and evolution: is our understanding of genetics sufficient to explain evolution? J Anim Breed Gen 1993 110: 161-170.
57. Lindqvist C. Domestication effects on foraging behaviour – consequences for adaptability in chickens. Linköping Studies in Science and Technology. 2008, Dissertation No. 1164.
58. De Vries MJ, Veerkamp RF. Energy balance of dairy cattle in relation to milk production variables and fertility. J Dairy Sci 2000; 83: 62-69.
59. Butler WR. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livest Prod Sci 2003; 83: 211-218.
60. Llewellyn S, Fitzpatrick R, Kenny DA, Murphy JJ, Scaramuzzi RJ, Wathes DC. Effect of negative energy balance on the insulin-like growth factor system in pre-recruitment ovarian follicles of post partum dairy cows. Reproduction 2007; 133: 627-639
61. Leroy JL, Vanholder T, Mateusen B, Christophe A, Opsomer G, de Kruif A, Genicot G, van Soom A. Non-sterified fatty acids in follicular fluid of dairy cows and their effect on developmental capacity of bovine oocytes in vitro. Reproduction 2005; 130: 485-495.
62. Vanholder T, Leroy JL, van Soom A, Opsomer G, Maes D, Coryn M, de Kruif A. Effect of non-esterified fatty acids on bovine granulosa cell steroidogenesis and proliferation in vitro. Anim Reprod Sci 2005; 87: 33-44.
63. Patton J, Kenny DA, McNamara S, Mee JF, O'Mara FP, Diskin MG, Murphy JJ. Relationships among milk production, energy balance, plasma analyses, and reproduction in Holstein-Friesian cows. J Dairy Sci 2007; 90: 649-658.
64. Alban L, Agger JF. Welfare in Danish dairy herds 1. Disease management routines in 1983 and 1994. Acta vet Scand 1996; 37: 49-63.
65. Alban L, Agger JF. Welfare in Danish dairy herds 2. Housing systems and grazing procedures in 1983 and 1994. Acta vet Scand 1996; 37: 65-77.
66. Platz S, Ahrens F, Bendel J, Meyer HH, Erhard MH. What happens with cow behaviour when replacing concrete slatted floor by rubber coating: A case study. J Dairy Sci 2008; 91: 999-1004.
67. Bergsten C. Effects of conformation and management system on hoof and leg diseases and lameness in dairy cows. Vet Clin North Am Food Anim Pract 2001; 17: 1-23.
68. Roche JF. The effect of nutritional management of the dairy cow on reproductive efficiency. Anim Reprod Sci 2006; 96: 282-296.
69. Rukkwamsuk T, Kruip TA, Wensing T. Relationship between overfeeding and overconditioning in the dry period and the problems of high producing dairy cows during the postparturient period. Vet Q 1999; 21: 71-77.
70. Smith BI, Risco CA. Predisposing factors and potential causes of postpartum metritis in dairy cattle. Comp Contin Educ Pract Vet 2002; 24: S74-S80.
71. Stronge AJH, Sreenan JM, Diskin MG, Mee JF, Kenny DA, Morris DG. Post insemination milk progesterone concentration and embryo survival in dairy cows. Theriogenology 2005; 64: 1212-1224.
72. Al-Katanani YM, Webb DW, Hansen PJ, Factors affecting seasonal variation in 90-day nonreturn rate to first service in lactating Holstein cows in a hot climate. J Dairy Sci 1999; 82: 2611-2616.
73. Snijders SE, Dillon PG, O'Farrell KJ, Diskin M, Wylie AR, O'Callaghan D, Rath M, Boland MP. Genetic merit for milk production and reproductive success in dairy cows. Anim Reprod Sci 2001; 65:17-31.
74. Sartori R, Sartori-Bergfelt R, Mertens SA, Guenther JN, Parrish JJ, Wiltbank MC. Fertilization and early embryonic development in heifers and lactating cows in summer and lactating and dry cows in winter. J Dairy Sci 2002; 85: 2803-2812.
75. Rodriguez-Martinez H, Barth AD. *In vitro* evaluation of sperm quality related to *in vivo* function and fertility. In: Reproduction in Domestic Ruminants VI. Edited by JI Juengel, JF Murray, MF Smith. Nottingham University Press, Nottingham, UK, 2007; 39-54 (Soc Reprod

Fert 2007; 64: 39-54).

76. Ostermeier GC, Goodrich RJ, Moldenhauer JS, Diamond MP, Krawetz SA. A suite of novel human spermatozoal RNAs. J Androl 2005; 26: 70-74.
77. Miller D, Ostermeier GC. Spermatozoal RNA: Why is it there and what does it do? Gynecol Obstet Fertil 2006; 34: 840-846.
78. Lalancette C, Thibault C, Bachand I, Caron N, Bissonnette N. Transcriptome analysis of bull semen with extreme nonreturn rate: Use of suppression-substractive hybridization to identify functional markers for fertility. Biol Reprod 2008; 78: 618-635.
79. Roche JF, Mackey D, Diskin MD. Reproductive management of postpartum cows. Anim Reprod Sci 2000; 60-61: 703-712.
80. Lucy MC. Mechanisms linking nutrition and reproduction in postpartum cows. Reproduction 2003; S61: S415-S417.
81. Reksen O, Grohn YT, Havrevoll O, Bolstad T, Waldmann A, Ropstad E. Relationships among milk progesterone, concentrate allocation, energy balance, milk yield and conception rate in Norwegian cattle. Anim Reprod Sci 2002; 73: 169-184.
82. Inskeep EK. Preovulatory, postovulatory, and postmaternal recognition effects of concentrations of progesterone on embryonic survival in the cow. J Anim Sci 2004; 82: E24-E39.
83. Lopez H, Satter LD, Wiltbank MC. Relationship between level of milk production and estrous behavior of lactating dairy cows. Anim Reprod Sci 2004; 81: 209-223.
84. Vanholder T, Opsomer G, de Kruif A. Aetiology and pathogenesis of cystic ovarian follicles in dairy cattle: a review. Reprod Nutr Dev 2006; 46: 105-119.
85. Wiltbank M, Lopez H, Sartori R, Sangsritavong S, Gümen A. Changes in reproductive physiology of lactating dairy cows due to elevated steroid metabolism. Theriogenology 2006; 65: 17-29.
86. Båge R, Gustafsson H, Larsson B, Forsberg M, Rodriguez-Martinez H. Repeat breeding in dairy heifers: follicular dynamics and oestrous cycle characteristics in relation to sexual hormone patterns. Theriogenology 2002; 57: 2257-2269.
87. Grimard B, Freret S, Chevallier A, Pinto A, Ponsart C, Humblot P. Genetic and environmental factors influencing first service conception rate and late embryonic/foetal mortality in low fertility dairy herds. Anim Reprod Sci 2006; 91: 31-44.
88. Båge R, Petyim S, Larsson B, Hallap T, Bergqvist AS, Gustafsson H, Rodriguez-Martinez H. Oocyte competence in repeat-breeder heifers: effects of an optimized ovum pick-up schedule on expression of oestrus, follicular development and fertility. Reprod Fertil Dev 2003; 15: 115-123.
89. Hansen PJ, Soto O, Natzke RP. Mastitis and fertility in cattle – possible involvement of inflammation or immune activation in embryonic mortality. Am J Reprod Immun 2004; 51: 294-301.
90. Leroy JL, Opsomer G, Van Soom A, Goovaerts IG, Bols PE. Reduced fertility in high-yielding dairy cows: Are the oocyte and embryo in danger? Part I - Importance of negative energy balance and altered corpus luteum function to the reduction of oocyte and embryo quality in high-yielding dairy cows. Reprod Domest Anim 2008; 43: (In press).
91. Khatib H, Malteca C, Monson RL, Schutzkus V, Wang X, Rutledge JJ. The fibroblast growth factor 2 gene is associated with embryonic mortality in cattle. J Anim Sci 2008; (in press).
92. Petersson K-J, Gustafsson H, Strandberg E, Berglund B. Atypical progesterone profiles and fertility in Swedish dairy cows. J Dairy Sci 2006; 89: 2529-2538.
93. Dobson H, Tebble JE, Smith RF, Ward WR. Is stress really all that important? Theriogenology 2001; 55: 65-73.
94. Zavy MT, Geisert RD. Embryonic mortality in domestic species. CRC Press, Boca Raton, USA, 1994.
95. Butler WR. Review: effect of protein nutrition on ovarian and uterine physiology in dairy cattle. J Dairy Sci 1998; 81: 2533-2539.
96. López-Gatius F, Santolaria P, Yániz J, Garbayo JM, Hunter RHF. Timing of early fetal loss for single and twin pregnancies in dairy cattle. Reprod Dom Anim 2004; 39: 429-433.
97. López-Gatius F, Garbayo JM, Santolaria P, Yániz J, Ayad A, de Sousa NM, Beckers JF. Milk production correlates negatively with plasma levels of pregnancy-associated glycoprotein (PAG) during the early fetal period in high producing dairy cows with live fetuses. Dom Anim

Endocrinol 2007; 32: 29-42.
98. Jousan FD, Drost M, Hansen PJ. Factors associated with early and mid-to late fetal loss in lactating and nonlactating Holstein cattle in a hot climate. J Anim Sci 2005; 83: 1017-1022.
99. García-Ispierto I, López-Gatius F, Santolaria P, Yäniz JL, Nogareda C, López-Béjar M, De Rensis, F (2006) Relationship between heat stress during the peri-implantation period and early fetal loss in dairy cattle. Theriogenology 2006; 65: 799-807.
100. Silke V, Diskin MG, Kenny DA, Boland MP, Dillon P, Mee JF, Sreeenan JM. Extent, pattern and factors associated with late embryonic loss in dairy cows. Anim Reprod Sci 2002; 71: 1-12.
101. López-Gatius F, Yániz J, Madriles-Helm D. Effects of body condition score and score change on the reproductive performance of dairy cows: a meta-analysis. Theriogenology 2003; 59: 801-812.
102. Santos J, Thatcher W, Chebel R, Cerri R, Galvao K. The effect of embryonic death rates in cattle on the efficacy of oestrus synchronization programs. Anim Reprod Sci 2004; 82-83: 513-535.
103. Trasler JM. Gamete imprinting: setting epigenetic patterns for the next generation. Reprod Fertil Dev 2006; 18: 63-69.
104. Oishi M, Gohma H, Hashizume K, Taniguchi Y, Yasue H, Takahashi S, Yamada T, Sasaki Y. Early embryonic death-associated changes in genome-wide gene expression profiles in the fetal placenta of the cow carrying somatic nuclear-derived cloned embryo. Mol Reprod Dev 2006; 73: 404-409.
105. Constant F, Guillomot M, Heyman Y, Vignon X, Laigre P, Servely JL, Renard JP, Chavatte-Palmer P. Large offspring or large placenta syndrome? Morphometric analysis of late gestation bovine placentomes from somatic nuclear transfer pregnancies complicated by hydrallantois. Biol Reprod 2006; 75: 122-130.
106. Kremenskoy M, Kremeska Y, I M, Imai K, Takahashi S, Hashizume K, Yagi S, Shiota K. DNA methylation profiles of donor nuclei cells and tissues of cloned bovine fetuses. J Reprod Dev 2006; 52: 259-266.
107. Hall VJ, Ruddock NT, French AJ. Expression profiling of genes crucial for placental and preimplantation development in bovine in vivo, in vitro, and nuclear transfer blastocysts. Mol Reprod Dev 2005; 72: 16-24.
108. Berry DP, Lee JM, MacDonald KA, Roche JR. Body condition score and body weight effects on dystocia and stillbirths and consequent effects on post calving performance. J Dairy Sci 2007; 90: 4201-4211.
109. Bicalho RC, Galvao KN, Cheong SH, Gilbert RO, Warnik LD, Guard CL. Effect of stillbirths on dam survival and reproduction performance in Holstein dairy cows. J Dairy Sci 2007; 90: 2797-2803.
110. Sewalem A, Miglior F, Kistemaker GJ, Sullivan P, Van Doormaal BJ. Relationship between reproduction traits and functional longevity in Canadian dairy cattle. J Dairy Sci 2008; 91: 1660-1668.
111. Berglund B, Philipsson J. Increasing stillbirth rates in the Swedish Friesian population. 43rd Ann Meet EAAP, Madrid, Spain, 992.
112. Heringstad B, Chang YM, Svendsen M, Gianola D. Genetic analysis of calving difficulty and stillbirth in Norwegian Red cows. J Dairy Sci 2007; 90: 3500-3507.
113. Kuhn C, Bennewitz J, Reinsch N, Xu N, Thomsen H, Looft C, Brockmann GA, Schwerin M, Weimann C, Hiendleder S, et al. Quantitative trait loci mapping of functional traits in the German Holstein cattle population. J Dairy Sci 2003; 86: 360-368.
114. Kornmatitsuk B, Dahl E, Ropstad E, Beckers JF, Gustafsson H & Kindahl H. Endocrine profiles, haematology and pregancy outcomes of late pregnant Holstein dairy heifers sired by bulls giving a high or low incidence of stillbirth. Acta vet Scand 2004; 45: 47-68.
115. Lidfors L, Berg C, Algers B. Integration of natural behavior in housing systems. Ambio 2005; 34: 325-330.
116. Lucy MC. Fertility in high-producing dairy cows: Reasons for decline and corrective strategies for sustainable improvement. In: Reproduction in Domestic Ruminants VI. Edited by JI Juengel, JF Murray and MF Smith. Nottingham University Press, Nottingham, UK, 2007; 64: 237-254 (Soc Reprod Fert 2007; 64: 237-254).

117. Thatcher WW, Bilby TR, Bartolome JA, Silvestre F, Staples CR, Santos JE. Strategies for improving fertility in the modern dairy cow. Theriogenology 2006; 65: 30-44.
118. Garnsworthy PC. Body condition score in dairy cows: targets for production and fertility. In: Recent advances in animal nutrition (Garnsworthy PC & Wiseman J, eds), 2006: 61-86, Nottingham University Press, Notingham, UK.
119. Yan T, Mayne CS, Keady TWJ, Agnew RE. Effects of dairy cow genotype with two planes of nutrition on energy partitioning between milk and body tissue. J Dairy Sci 2006; 89: 1031-1042.
120. Schei I, Volden H, Baevre L. Effects of energy balance and metabolizable protein level on tissue mobilization and milk performance of dairy cows in early lactation. Liv Prod Sci 2005; 95: 35-47.
121. Reynolds CK. Production and metabolic effects of site of starch digestion in dairy cattle. Anim Feed Sci & Technol 2006; 130: 78-94.
122. Castañeda-Gutierrez A, Overton TR, Butler WR, Bauman DE. Dietary supplements of two doses of calcium salts of conjugated linoleic acid during the transition period and early lactation. J Dairy Sci 2005; 88: 1078-1089.
123. Butler ST, de Veth MJ. Effects of CLA supplementation on milk production and reproductive performance of dairy cows. Proc. Int Conf on Fertility in dairy cows, Liverpool Hope University, UK, 30-31 August 2007 (EAAP Satellite Meeting), 2007; 1: 20.
124. Webb R, Garnsworthy PC, Campbel BK, Hunter MG. Intra-ovarian regulation of follicular development and oocyte comptetence in farm animals. Theriogenology 2007; 68 (Suppl 1): S22-29.
125. Miyoshi S, Pate JL, Palmquist DL. Effects of propylene glycol drenching on energy balance, plasma glucose, plasma insulin, ovarian function and conception in dairy cows. Anim Reprod Sci 2001; 68: 29-43.
126. Gong JG, Lee WJ, Garnsworthy PC, Webb R. Effect of dietary-induced increases in circulating insulin concentrations during the early postpartum period on reproductive function in dairy cows. Reproduction 2002; 123: 419-427.
127. Pehrson B, Plym-Forshell K, Carlsson J. The effect of additional feeding on the fertility of high yielding dairy cows. Zbl Vetmed A 1992; 39: 187-192.
128. Fouladi-Nashta AA, Gutierrez CG, Gong JG, Garsworthy PC, Webb R. Impact of dietary fatty acids on oocyt quality and development in lactating dairy cows. Biol Reprod 2007; 77: 9-17.
129. Lemley CO, Butler ST, Butler WR, Wilson ME. Short communication: insulin alters hepatic progesterone catabolic enzymes cytochrome P450 2C and 3A in dairy cows.). J Dairy Sci 2008; 91: 641-645.
130. Leroy JL, Opsomer G, Van Soom A, Goovaerts IG, Bols PE. Reduced fertility in high-yielding dairy cows: Are the oocyte and embryo in danger? Part II – Mechanisms linking nutrition and reduced oocyte and embryo quality in high-yielding dairy cows. Reprod Domest Anim 2008; 43: (In press).
131. Butler ST. Conjugated linoleic acids and reproductive performance of dairy cows. Proc. Int Conf on Fertility in dairy cows, Liverpool Hope University, UK, 30-31 August 2007 (EAAP Satellite Meeting), 2007; 1: 22.
132. Garnsworthy PC, Fouladi-Nashta AA, Mann GE, Sinclair KD, Armstrong DG, Gong JG, Gutierrez CG, Webb R. How does nutrition interact with fertility in dairy cows? Cattle Practice 2008; 16 (Part 1): 20-23.
133. Zwald NR, Weigal KA, Chang YM, Welper RD, Clay JS. Genetic selection for health traits using producer-recorded data. I. Incidence rates, heritability estimates and sire breeding values. J Dairy Sci 2004; 87: 4287-4294.
134. Retnayake DRTG, Berglund B, Bertilsson J, Forsberg M, Gustafsson H. Fertility in dairy cows managed for calving intervals of 12, 15 or 18 months. Acta vet scand 1998; 39: 215-228.
135. Kadokawa H, Martin GB. A new perspective on management of reproduction in dairy cows: the need for detailed metabolic information, an improved selection index and extended lactation. J Reprod Dev 2006; 52:161-168.
136. Bertilsson J, Berglund B, Ratnayake G, Svennersten-Sjaunja K, Wiktorsson H. Optimising lactation cycles for the high-yielding dairy cow. A European perspective. Livestock Prod Sci 1997; 50: 5-13.

137. Sørensen A, Muir DD, Knight CH. Extended lactation in dairy cows: effects of milking frequency, calving season and nutrition on lactation persistency and milk quality. J Dairy Res 2008; 75: 90-97.
138. Patton J, Kenny DA, Mee JF, O'Mara FP, Wathes DC, Cook M, Murphy JJ. Effect of milking frequency and diet on milk production, energy balance, and reproduction in dairy cows. J Dairy Sci 2006; 89: 1478-1487.
139. Heins BJ, Hansen LB, Seykora AJ, Johnsson DG, Linn JG, Romano JE, Hazel AR. Crossbreds o Jersey x Holstein compared to pure Holsteins for production, fertility, and body and udder measurements during first lactation. J Dairy Sci 2008; 91: 1270-1278.
140. Heins BJ, Hansen LB, Seykora AJ. Production of pure Holsteins versus crossbreds of Holstein with Normande, Montbeliarde, ans Scandinavian Red. J Dairy Sci 2006b; 89: 2799-2804.
141. Heins BJ, Hansen LB, Seykora AJ, Hazel AR, Linn JG, Johnson DG, Hansen WP. Crossbreds of Jersey/Holstein compared to pure Holsteins for production, calving difficulty, stillbirths, and fertility. J Dairy Sci 2006a; 89 (Suppl 1): A245-246.
142. Løvendahl P, Chagunda MGG. Assessment of fertility in dairy cows based on electronic monitoring of their physical activity. 8th WCGALP, Belo Horizonte, Brazil. Proc. Commun. 2006; 1: 18-04 (http://www.wcgalp8.org.br).
143. Royal MD, Flint APF, Wooliams JA. Genetic and phenotypic re1ationships among endocrine and traditional fertility traits and production traits in Holstein-Friesian dairy cows. J Dairy Sci 2002; 85: 958-967.
144. Royal MD, Pryce JE, Wooliams JA, Flint APF. The genetic relationship between commencement of luteal activity and calving interval, body condition score, production, and linear type traits in Holstein-Friesian dairy cattle. J Dairy Sci 2002b; 85: 3071-3080.
145. Petersson K-J, Berglund B, Strandberg E, Gustafsson H, Flint APF, Woolliams JA, Royal MD. Genetic analysis of postpartum measures of luteal activity in dairy cows. J Dairy Sci 2007; 90: 427-434.
146. Petersson KJ, Strandberg E, Gustafsson H, Royal MD, Berglund B. Detection of delayed ciclicity in dairy cows based on progesterone content in monthly milk samples. Prev Vet Med 2008; 86: 153-163.
147. Steine G, Kristofersson D, Guttormsen AG. Economic evaluation of the breeding goal for Norwegian Red dairy cattle. J Dairy Sci 2008; 91: 418-426.
148. Nielsen HM, Christensen LG, Ödegård J. A method to define breeding goals for sustainable dairy cattle production. J Dairy Sci 2006; 89: 3615.3625.
149. Van der Lende T, Kaal L, Roelofs RMG, Veerkamp RF, Schrooteb C, Bovenhuis H. Infrequent milk progesterone measurements in daughters enable bull selection for cow fertility. J Dairy Sci 2004; 87: 3953-3957.
150. Veerkamp RF, Brotherstone S. Genetic correlations between linear type traits, food intake, live weight and condition score in Holstein Friesian dairy cattle. Anim Sci 1997; 64: 385-392.
151. Pryce JE, Coffey MP, Brotherstone SH, Woolliams JA. Genetic relationships between calving interval and body condition score conditional on milk yield. J Dairy Sci 2002; 85: 1590-1595.
152. De Haas Y, Janss LL, Kadarmideen HN. Genetic correlations between body condition scores and fertility in dairy cattle using bivariate random regression models. J Anim Breed Genet 2007; 124: 277-285.
153. De Jong G. Usage of predictors for fertility in the genetic evaluation application in The Netherlands. Interbull bull 2005; 33 69-73.
154. Pryce JE, Harris BL. Genetics of body condition score in New Zealand dairy cows. J Dairy Sci 2006; 89: 4424-4432.
155. Robinson JJ, Ashworth CJ, Rooke JA, Mitchell LM, McEvoy TG. Nutrition and fertility in ruminant livestock. Anim –Feed Sci Technol 2006; 126: 259-276.
156. Schrooten C, Bink MC, Bovenhuis H. Whole genome scan to detect chromosomal regions affecting multiple traits in dairy cattle. J Dairy Sci 2004; 87:3550-3560.
157. Kappes SM, Bennett GL, Keele JW, Echternkamp SE, Gregory KE, Thallman RM. Initial results of genomic scans for ovulation rate in a cattle population selected for increased twinning rate. J Anim Sci 2000; 78: 3053-3059.
158. Schneider F, Tomek W, Grundker C. Gonadotropin-releasing hormone (GnRH) and ist natural analogues: a review. Theriogenology 2006; 66: 691-709.

159. Fowden AL, Sibley C, Reik W, Constancia M. Imprinted genes, placental development and fetal growth. Horm Res 2006; 65: 50-58.
160. Hastings N, Donn S, Derecka K, Flint AP, Woolliams JA. Polymorphisms within the coding region of the bovine luteinizing hormone receptor gene and their association with fertility traits. Anim Genet 2006; 37: 583-585.
161. Van der Beek S. Effect of genomic selection on national and international genetic evaluations. Interbull bull 2007; 37: 115-118.
162. Van Raden PM. Genomic measures of relationship and inbreeding. Interbull bull 2007; 37: 33-36.
163. Meuwissen THE, Hayes BJ, Goddard ME. Prediction of total genetic value using genome-wide dense marker maps. Genetics 2001; 157: 1819-1829.
164. Schaeffer LR. Strategy for applying genome-wide selection in dairy cattle. J Anim Breed Genet 2006; 123: 218-223.

Hrm/2008

Abbreviations

AI	artificial insemination
AM	animal model
BC	body condition
BCS	body condition score
BHB	beta-hydroxy butyrate
bLH	bovine luteinizing hormone
BLUP	best linear unbiased prediction
CI	calving interval
CLA	conjugated linoleic acid (trans-10, cis-12 CLA)
CR	conception rate
DIY-AI	do-it-yourself-AI
DMI	dry matter intake
DNA	deoxyribo-nucleic acid
ECM	energy-corrected milk
EU	European Union
FGF2	fibroblast growth factor-2
GH	growth hormone
GnRH	gonadotropin-releasing hormone
IGFBP-2	insulin-growth factor binding protein-2
IFTN	interferon-tau
IGF-I/II	insulin-growth factor-I or II
Interbull	International Bull Evaluation Service
LDH	lactate dehydrogenase
LH	luteinizing hormone
LW	live weight
ML	metabolic load
MS	metabolic stress
mRNA	messenger RNA
NEB	negative energy balance
NEFA	non-esterified fatty acids
NRR	non-return to estrus rate
OR	odd ratios
PAG	pregnancy-associated glycoproteins
P_4	progesterone
$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$
PR	pregnancy rate
QTL	quantitative trait loci
rbST	recombinant bovine somatotropin
RFM	retained fetal membranes
RNA	ribo-nucleic acid
SH	Swedish Holstein breed
SME	small and medium enterprise
SNP	single nucleotide polymorphisms
SR	Swedish Red breed (earlier known as SRB: Swedish Red and White breed)
TDM	test day model
TMR	total mixed ration