

## Pregnancy losses in cattle: potential for improvement

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**Abstract.** For heifers, beef and moderate-yielding dairy cows, it appears that the fertilisation rate generally lies between 90% and 100%. For high-producing dairy cows, there is a less substantive body of literature, but it would appear that the fertilisation rate is somewhat lower and possibly more variable. In cattle, the major component of embryo loss occurs in the first 16 days following breeding (Day 0), with emerging evidence of greater losses before Day 8 in high-producing dairy cows. In cattle, late embryo mortality causes serious economic losses because it is often recognised too late to re-breed females. Systemic concentrations of progesterone during both the cycle preceding and following insemination affect embryo survival, with evidence of either excessive or insufficient concentrations being negatively associated with survival rate. The application of direct progesterone supplementation or treatments to increase endogenous output of progesterone to increase embryo survival cannot be recommended at this time. Energy balance and dry matter intake during the first 4 weeks after calving are critically important in determining pregnancies per AI when cows are inseminated at 70–100 days after calving. Level of concentrate supplementation of cows at pasture during the breeding period has minimal effects on conception rates, although sudden reductions in dietary intake should be avoided. For all systems of milk production, more balanced breeding strategies with greater emphasis on fertility and feed intake and/or energy must be developed. There is genetic variability within the Holstein breed for fertility traits, which can be exploited. Genomic technology will not only provide scientists with an improved understanding of the underlying biological processes involved in fertilisation and the establishment of pregnancy, but also, in the future, could identify genes responsible for improved embryo survival. Such information could be incorporated into breeding objectives in order to increase the rate of genetic progress for embryo survival. In addition, there is a range of easily adoptable management factors, under producer control, that can either directly increase embryo survival or ameliorate the consequences of low embryo survival rates. The correction of minor deficits in several areas can have a substantial cumulative positive effect on herd reproductive performance.

**Additional keywords:** cow, embryonic mortality, fertilisation, progesterone.

### Introduction

Embryo mortality is a major cause of economic loss in dairy production systems. Direct effects of embryonic mortality are reflected in reduced conception rates with consequent effects for efficiency of production and profitability. In dairying, the increase in milk yield observed over the past 50 years has been accompanied by a decline in cow fertility in both high-input total mixed ration (TMR)-based and less-intensive pasture-based systems (for a review, see Diskin *et al.* 2011), with evidence now emerging that the decline has at least stopped and a phenotypic improvement in cow fertility is being observed. The inclusion of fertility traits or proxies for such traits in breeding indices has partially contributed to halting the decline in fertility and evidence of improvements in conception rates and related traits (Barbat *et al.* 2010), although improved management and nutrition are likely to be the more important factors. The

objective of this paper is to review current information on embryonic and early fetal mortality in cattle and to propose potential avenues to minimise such losses.

### Fertilisation rate in cattle

In heifers and in moderate-yielding dairy cows, fertilisation rates are of the order of 90%–100% following the use of high-quality semen (for a review, see Sreenan and Diskin 1986). In contrast, there is less quantitative information on fertilisation rate for higher-producing dairy cows. Wiebold (1988), using a non-surgical embryo recovery technique on Day 7 following oestrus, recovered 25 ova or embryos from 23 lactating cows, with all recovered ova having been fertilised. Ryan *et al.* (1993), in a study on the effects of ambient temperature on fertilisation rate, reported no effect of temperature and quoted fertilisation

**Table 1.** Projected reproductive outcomes for low-yielding dairy (typical of pasture-based systems) cows and heifers compared with high-yielding dairy cows (typical of intensive grain or total mixed ration-based systems) dairy cows

	Low-yielding cows and heifers	High-yielding dairy cows	Comments
No. inseminated	100	100	
No. with fertilised ovum at Day 4	90	85	
No. pregnant at Day 28	60	45	
No. pregnant at Day 90	56	36	Based on 7% and 20% losses of pregnancies in low- and high-yielding cows, respectively, between Days 28 and 90
No. calving	54	34	Assumes normal loss rates for the last 6 months of gestation

rates of 82.4% and 79.5% for high and low temperatures, respectively. Sartori *et al.* (2002) recorded a fertilisation rate of 55.6% in lactating dairy cows compared with 100% for heifers under high ambient temperatures, whereas in a subsequent study during the cool season fertilisation rates were 87.8% and 89.5% for lactating and non-lactating dairy cows, respectively. In two recent studies from California (Cerri *et al.* 2009a, 2009b), fertilisation rates of 83% and 88% were recorded in high-producing dairy cows (herd averages >11 600 kg milk). Based on published results, it appears that fertilisation rate may be a little lower in high- and moderate-producing dairy cows, at least where the potential for heat stress exists.

### Extent and timing of early embryo loss in cattle

Sreenan and Diskin (1986) calculated an embryonic and fetal mortality rate (excluding fertilisation failure) of approximately 40% for moderate-producing cows based on a fertilisation rate of 90% and an average calving rate of approximately 55%, with an estimated 70%–80% of losses sustained between Days 8 and 16 after AI. The comparative figure for high-producing dairy cows, based on a fertilisation rate of 90% and a calving rate of 40%, is 56%. Evidence is now emerging that the pattern of early embryo death in the modern high-producing cow may be different to that observed in heifers and lower-yielding dairy cows. For example, in the study of Sartori *et al.* (2002), conducted during the summer in Wisconsin, a significantly greater proportion of embryos recovered on Days 6 and 7 were of higher-quality grade from heifers (72%) compared with lactating cows (33%). Furthermore, during the winter component of the same study, a greater proportion of high-quality grade embryos were recovered from dry (83%) than lactating (53%) cows. This trend towards comparatively higher recovery of low-quality, abnormal or retarded embryos from high-yielding dairy cows is also evident in the study of Wiebold (1988), who found that 52% of the embryos from high-yielding dairy cows on Days 6 or 7 were abnormal, based on morphological criteria. Ryan *et al.* (1993) reported that the proportions of high-quality grade embryos recovered under conditions of high (58.5%) or low (51.6%) ambient temperature were similar. In two recent studies from California (Cerri *et al.* 2009a, 2009b), 59%–89% of recovered embryos were classified as Grades 1 and 2 on Day 6. A feature of these recent studies is the consistent evidence that a high proportion (range 11%–67%) of embryos recovered on Days 6 to 7 after oestrus from moderate- or high-producing dairy cows

were classified as abnormal, in contrast with a much lower incidence (range 17%–28%) in heifers and non-lactating cows (6%; average of four studies) reported by Sreenan and Diskin (1986). It would appear that early embryo loss is greater in the modern high-producing dairy cow and that a much higher proportion of the embryos die before Day 7 following AI compared with lower-producing cows or heifers. When embryo survival extends beyond the stage of maternal recognition of pregnancy, luteal regression is delayed, resulting in a delayed return to oestrus. Humblot (2001) suggested that returns to oestrus after Day 24 could reflect embryonic losses occurring after Day 16 of gestation. There is a lack of precise information on the extent of embryo losses after Day 16. Based on the published evidence, the expected outcome of 100 inseminations of British Friesian and Holstein–Friesian cows is summarised in Table 1.

### Late embryo and fetal loss in cattle

Silke *et al.* (2002) quantified embryo/fetal loss from Days 28 to 84 of gestation in 1046 lactating dairy cows and 162 dairy heifers managed on pasture-based systems of milk production. The overall rates and the pattern of loss between Days 28 and 84 of gestation, based on the proportions of those initially with a confirmed pregnancy at Day 28, were similar for cows producing an average 7247 kg milk and heifers (7.2% vs 6.1%, respectively). Almost half the total recorded loss (47.5%) occurred between Days 28 and 42 of gestation. In that study, no association was evident between level of milk production or milk energy output measured to Day 120 of lactation, milk fat concentration, milk protein concentration or milk lactose concentration and embryo/fetal loss rate. In addition, the extent and pattern of embryo/fetal loss were not related to either the genetic merit of the cow herself or her sire. A subsequent Irish study (Horan *et al.* 2004) recorded a similar overall late embryo/fetal loss rate of 7.5% between Days 30 and 67 of gestation in dairy cows managed under pasture-based systems of production. The extent of late embryo/fetal mortality recorded in these two pasture-based studies is much lower than that reported for more intensively managed higher-yielding cows (Vasconcelos *et al.* 1997; Santos *et al.* 2004), although the causes for the apparent difference are not clear. In a study involving 1285 Holstein cows in France, Grimard *et al.* (2006) recorded an overall incidence of late embryo death (defined as embryo/fetal death between Days 21 and 80 of gestation) of 25% and found that the incidence

was associated with period of AI, level of milk production and body condition score (BCS), but not the breeding value. Cunha *et al.* (2008) recorded lower pregnancy loss rates between Days 29 and 57 of gestation in cows with high compared with cows with low concentrations of progesterone during the growth of the follicular wave preceding ovulation and AI (6.8% vs 14.3%, respectively). Other studies that have used controlled breeding systems have recorded increased pregnancy loss rates in anovulatory compared with cyclic dairy cows (Santos *et al.* 2004; Sterry *et al.* 2006; Stevenson *et al.* 2006). This would suggest that elevated concentrations of progesterone before AI have a positive carryover effect on the maintenance of pregnancy even well after the commencement of implantation. Based on the published evidence, it would appear that the extent of late embryo/fetal losses is approximately twice as high in high-yielding cows compared with lower-yielding cows and heifers managed on pasture-based systems, with the actual magnitude and consequences of such pregnancy losses, as a proportion of all losses, much greater in higher-yielding cows. This is due to the typically lower pregnancy rates (Day 28–30) in high-producing cows (~35%–45%) compared with their lower-yield counterparts (55%–65%).

Although the extent of late embryo loss is numerically lower than early embryo mortality, it causes serious economic losses in all production systems, but particularly in seasonal calving herds because it is often detected after the end of the season breeding period resulting in increased culling rates.

### Causes of embryo or fetal loss

#### Genetic

With the development of high-throughput, high-density single nucleotide polymorphism (SNP) genotyping and indeed whole-genome sequencing, our understanding of the genetic causes of infertility has improved considerably in the recent years and this is likely to accelerate in the coming decade. Indeed, the availability of commercial SNP genotyping assays now facilitates the selection of animals carrying either superior or deleterious genetic variants for reproductive traits, thus enabling earlier and more accurate identification.

Lefebvre *et al.* (2011) identified three quantitative trait loci (QTL) for non-fertilisation or early embryo development in cattle, three QTL for late embryo mortality rate, eight QTL for total embryo mortality rate and three QTL for abortion rate. Bovine haplotypes have been shown to be associated with negative effects on fertility in the Brown Swiss, Holstein and Jersey breeds (VanRaden *et al.* 2011). More recently, McClure *et al.* (2014) identified a potentially causative mutation contained within three of these haplotypes (Brown Swiss Haplotype 1 and Holstein Haplotypes 2 and 3). This is a non-synonymous SNP (T/C) within exon 24 of the structural maintenance of chromosomes 2 (*SMC2*) gene on chromosome 8, possibly a causative mutation resulting in defective embryo development in cattle. Data from our own laboratory have shown that SNPs in genes of the somatotrophic axis are associated with fertility in dairy cattle (Mullen *et al.* 2010, 2011; Waters *et al.* 2011, 2012), thus reinforcing the central role of the somatotrophic axis as a mediator of reproductive efficiency in cattle.

Furthermore, in a recent review Valour *et al.* (2015) summarised the effects of lethal mutations affecting *in utero* development in cattle. These mutations were recorded across the main dairy breeds. A significant negative association on calving rate was recorded for 20 haplotypes. Six regions were further investigated using whole-genome sequencing data from heterozygous bull carriers and strong candidate causative mutations were detected in six genes, including *FANCI* gene, the causative gene for brachyspina. In the cattle population, it would appear that the incidence of brachyspina is rare, at approximately <1 in  $10^5$  (Charlier *et al.* 2012). However, using high-density SNP arrays and next-generation sequencing, Charlier *et al.* (2012) observed a carrier frequency of 7.4% in the Holstein–Friesian breed. They demonstrated that the apparent discrepancy between the recorded population carrier frequency and lower observed population incidence of brachyspina was due to the *in utero* death of a high proportion of the homozygous fetuses.

The 1/29 Robertsonian chromosomal translocation (Gustavsson 1979) present in several beef breeds and the Scandinavian Red breeds (but not in the Holstein breed) has been suggested as a cause of reduced fertility of males and females heterozygous for this translocation. Sweden has successfully implemented a testing program to eliminate AI bulls that are carriers for this condition, resulting in improvement in fertility (Gustavsson 1979). In the Holstein breed, two major recessive defects affecting embryo/fetal survival have been detected. Deficiency of uridine monophosphate synthase (DUMPS; Robinson *et al.* 1984), a homozygous recessive condition, causes fetal death at 40–50 days gestation (Shanks and Robinson 1989). Testing of AI sires for DUMPS has reduced the frequency of heterozygous sires and homozygous recessive embryos, and has now almost eliminated this as a cause of infertility (VanRaden and Miller 2006). Complex vertebral malformations (CVM) is a lethal recessive condition that causes late fetal death in cattle. Prior to this, the defect was disseminated through the widespread use of the Holstein maternal sire of sires, Carlin-M Ivanhoe Bell. In a Swedish study, Berglund *et al.* (2004) recorded that carrier bulls had significantly lower breeding values for 168-day non-return rate than non-carriers but were not different at 28 and 56 days, indicating that the condition primarily affects late rather than early fetal mortality. Again, it is possible to greatly reduce the incidence of carriers by testing sires. Breeding companies should ensure that both the sire and dam of future AI bulls are not carriers of genes with known adverse effects on cow fertility. The availability of high-throughput high-density SNP genotyping and whole-genome sequencing now ensures accurate parentage determination, with the culling of bulls that are carriers for genes with adverse effects on fertility an imperative.

#### Genetic improvement of embryo survival

Berry *et al.* (2014a), using meta-analysis of published data, calculated mean heritability estimates of 0.021 to 0.034 for traits such as number of services, pregnancies per AI to first service, pregnancies per AI within a given period and non-return rate, which are all indirectly reflective of embryo survival rate. These low heritability estimates do not imply that genetic selection

cannot alter phenotypic performance, as evidenced by the recorded decline in fertility parameters in dairy cows up until approximately 10 years ago, which was partly attributable to intensive selection for milk production, and the slight reversal of that trend in recent years since the development of more broad-based breeding objectives and the inclusion of fertility variables in dairy cow genetic indices. Genomic selection that provides estimates of the genetic merit of an animal based solely on their DNA has become a reality with the commercial availability of high-density SNP arrays. The biggest impact of genomic selection has been to increase the reliability of breeding values without animal or progeny records, particularly for low-heritability traits and traits that are difficult to measure. Genomic information is now included in most advanced dairy cattle breeding programs, with clear evidence of the benefit of its inclusion (Berry *et al.* 2014a). Because the genetic correlation between fertility and production traits is not unity, it is possible to simultaneously improve both. Other advanced breeding tools combined with new knowledge from the ‘-omics’ biology (for a review, see Berry *et al.* 2014a) will offer new opportunities to improve cow reproductive performance by genetic means.

### Inbreeding

The rate of inbreeding in most dairy cow populations is increasing by +0.10%–0.25% per annum (for a review, see Berry *et al.* 2014a). Several reproductive traits are adversely affected by inbreeding. McParland *et al.* (2007) observed that calving interval increased by almost 9 days in animals with a 12.5% level of inbreeding. In addition, maternal inbreeding has been reported to decrease the 56- to 70-day non-return rates by between 1% (Wall *et al.* 2003) and 2% (Cassell *et al.* 2003) per 10% increase in inbreeding coefficient of the dam. Conversely, inbreeding of the embryo has been reported to reduce the 70-day non-return rate by 1% for each 10% increase in the level of inbreeding of the embryo (Cassell *et al.* 2003; VanRaden and Miller 2006). Genetic variation in embryo survival may be attributable to the genetic constitution of the embryo itself and/or the genetic differences among dams with regard to their ability to provide an appropriate intraovarian and uterine environment. Controlling the level of inbreeding can be a challenge, particularly given the significant international dimension to dairy cattle breeding and the relatively small number of sires used combined with ever-increasing herd size. The use of computer-based mating programs allows producers to maximise specific breeding objectives while constraining inbreeding of the expected progeny. Recently, Sun *et al.* (2013) developed more sophisticated mating programs using genomic data that aim to maximise heterozygosity.

### Maternal age

Overall embryo survival rates would appear to be largely similar in heifers, beef cows and low- to moderate-producing dairy cows, but embryo survival rate is lower in high-producing dairy cows. However, it is improbable that this is totally an age- or parity-related phenomenon and is more likely due to the direct and indirect consequences of milk production. Kuhn *et al.* (2006) suggest that heifer conception rate is at a maximum at

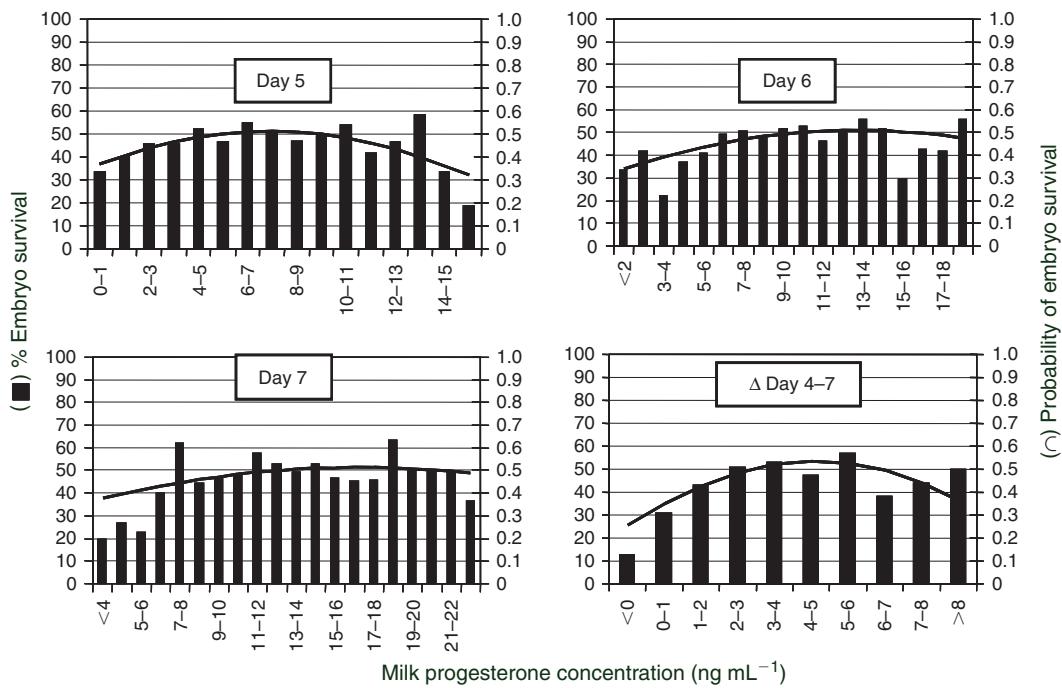
15–16 months of age. Breeding heifers at 26 months of age or older resulted in a 13% reduction in pregnancies per AI, presumably due to a lower embryo survival rate.

### Progesterone during the cycle immediately before AI and embryo survival rate

Concentrations of progesterone during the oestrous cycle preceding AI appear to have a key role in determining a successful outcome following insemination. Diskin *et al.* (2006) clearly showed that there is a positive linear association between the concentration of progesterone on the day of prostaglandin-induced luteolysis and subsequent embryo survival rate. A positive relationship has also been recorded between concentrations of progesterone 12 days before first AI and conception rate in lactating Holstein and Jersey cattle (Fonseca *et al.* 1983). These observations are also supported by the results of studies where systemic progesterone concentrations have been artificially manipulated by using controlled internal drug release (CIDR) devices in the cycle preceding insemination and have led to 5–7 percentage point improvements in pregnancy per AI (for a review, see Wiltbank *et al.* 2014). However, the improvement in pregnancy per AI is attributable to the elevation of circulating concentrations of progesterone induced by use of the CIDR and not because of differences in circulating concentrations of progesterone after AI. Cunha *et al.* (2008) recorded a lower pregnancy per AI at 29 days after AI in cows with lower concentrations of progesterone before AI compared with their counterparts that had high progesterone concentrations (37.1 vs 51.0, respectively;  $P < 0.001$ ). Therefore, it would appear that higher concentrations of progesterone during the growth follicular wave that produces the ovulatory follicle before AI not only produce a higher number of pregnancies at around Day 30 after AI, but also reduce the risk of subsequent loss of these pregnancies. Potential mechanisms by which low systemic concentrations of progesterone during the preceding oestrous cycle may reduce fertilisation and/or embryo survival rates include the production of oocytes that are at a more advanced stage of maturation at time of ovulation from persistent dominant follicles and increased frequency of LH pulses, which, in turn, induces increased secretion of  $17\beta$ -oestradiol or a change in endometrial morphology (for reviews, see Diskin *et al.* 2006; Wiltbank *et al.* 2014), altered follicular fluid composition and the possible premature development of pathways leading to uterine prostaglandin  $F_{2\alpha}$  secretion. (Cerri *et al.* (2011).

### Concentrations of progesterone around the time of AI

The development of Ovsynch-type controlled breeding programs has recently highlighted the importance of complete luteal regression and low concentrations of progesterone around the time of AI to achieve good subsequent conception rates (for a review, see Wiltbank *et al.* 2014). Wiltbank *et al.* (2014) concluded that circulating concentrations of progesterone greater than  $0.3$ – $0.5 \text{ ng mL}^{-1}$  were detrimental to conception rate in dairy cattle, most likely because of a reduced fertilisation rate when progesterone is elevated ( $>0.3 \text{ ng mL}^{-1}$ ), blocked ovulation or affected sperm transport. Possible strategies to induce more complete luteal regression and ensure lower



**Fig. 1.** Relationship between milk concentrations of progesterone on Days 5, 6 and 7 after AI and subsequent embryo survival rate in lactating dairy cows. (Reproduced with permission from Diskin *et al.* 2012.)

concentrations of progesterone may include increasing the dose of the prostaglandin analogue used, altering the timing of the prostaglandin treatment or administering a second dose of prostaglandin.

#### Postinsemination progesterone and embryo survival rate

Until recently, there was equivocation on the relationship between systemic concentrations of progesterone and embryo survival in cattle. Recent studies (Diskin *et al.* 2004; Stronge *et al.* 2005; Diskin *et al.* 2006; Parr *et al.* 2012) that examined the relationship between early and mid-luteal phase concentrations of progesterone and subsequent embryo survival per conception have used logistic regression techniques to model the relationship between the binomially distributed dependent variable (conception or embryo survival rate) and the continuously distributed independent variable (progesterone). Stronge *et al.* (2005) found a positive linear and quadratic relationship between concentrations of progesterone in milk on Days 5, 6 and 7 and the rate of change in concentrations of progesterone between Days 4 and 7 after insemination and embryo survival rate (Fig. 1). Further analysis of this dataset reveals that 75%, 72% and 56% of dairy cows have concentrations of progesterone that are optimal for embryo survival on Days 5, 6 and 7 after insemination, respectively. In both beef heifers (Diskin *et al.* 2006) and dairy heifers (Parr *et al.* 2012), a similar linear and quadratic association between peripheral concentrations of progesterone and embryo survival was observed. The effects of progesterone supplementation on embryo survival are equivocal. Starbuck *et al.* (2001) recorded an improvement in embryo survival rates for dairy cows following progesterone

supplementation of animals at risk of low embryo survival rate as a result of progesterone insufficiency. However, more recent studies have recorded no increase (Monteiro *et al.* 2014) or a decrease (56% vs 44%; Parr *et al.* 2014) in pregnancy per single AI following exogenous progesterone supplementation in dairy cows. Studies that have measured the effects of exogenous progesterone supplementation on embryo development have recorded a consistent increase in *in utero* blastocyst development rate and an increase in blastocyst interferon (IFN) gene expression and IFN- $\tau$  secretion, all of which could be hypothesised to benefit embryo survival. This highlights the apparent paradoxical effect of exogenous progesterone supplementation in terms of stimulating conceptus development but compromising the corpus luteum, ultimately leading to no net change in pregnancy per AI. Recent studies (Parr 2013) have provided new insights into the effects of exogenous progesterone during the early stages of the cycle following insemination. These studies showed that both the timing of initiation and duration of supplementation of exogenous progesterone had effects on circulating concentrations of progesterone, corpus luteum and embryo development in heifers with induced low progesterone in the early luteal phase. Embryo development was greater following supplemental progesterone treatments commencing on Day 4; however, a longer duration of supplemental progesterone had a negative effect on the development and lifespan of the corpus luteum. Such information is critical to the development of reliable progesterone supplementation strategies to increase embryo survival rate in cattle.

Further to the study of exogenous progesterone manipulation approaches, a series of experiments with dairy cows (Sangsrivavong *et al.* 2002) has clearly shown that peripheral

concentrations of progesterone and oestradiol are lowered by increased feed intake due to a higher hepatic metabolic clearance rate (MCR) of these steroids. From that study, it would appear that the MCR remains elevated in high-producing lactating dairy cows and that this, in turn, results in a lowering of peripheral concentrations of progesterone, thus increasing the risk of embryo death. Progesterone has also been shown to directly affect the growth and development of the bovine conceptus (Garrett *et al.* 1988) and to be positively correlated with IFN- $\tau$  secretion (Kerbler *et al.* 1997). McNeill *et al.* (2006) have shown that uterine expression of mRNA for progesterone and oestradiol receptors and retinol-binding protein mRNA is sensitive to changes in peripheral concentrations of progesterone during the first week after AI. Recently, Forde *et al.* (2009) reported that the transcriptome of the endometrium of cyclic heifers is sensitive to circulating concentrations of progesterone in the first few days after oestrus. Under low progesterone conditions, a suboptimal uterine environment with reduced ability to support conceptus elongation was observed.

As an alternative to direct exogenous progesterone supplementation, many studies have used human chorionic gonadotropin (hCG) or gonadotrophin-releasing hormone (GnRH) in attempts to increase circulating concentrations of progesterone and embryo survival rates. When hCG or GnRH is administered on Day 5 after AI, accessory corpora lutea are induced, with a concomitant increase in progesterone concentrations (Fricke *et al.* 1993). In the study of Nascimento *et al.* (2013a), 93% of dairy cows treated with hCG on Day 5 had induced accessory corpora lutea and increased circulating progesterone concentrations from Days 8 to 16. In a recent meta-analysis of 10 previous studies of the effects of hCG on subsequent fertility, there was a small increase in the conception rate of 3 percentage points (from 34.0% to 37.0%) in hCG-treated cows (Nascimento *et al.* 2013b). In a follow-on manipulative study, Nascimento *et al.* (2013b) recorded a 3.5 percentage point improvement (from 37.3% to 40.8%) following hCG administration. Interestingly, in that study all the effects of hCG on conception rates were recorded in primiparous (from 39.0% to 49.7%) but not multiparous (from 36.0% to 35.7%) cows (Nascimento *et al.* 2013b). Although the effects of hCG administration on pregnancies per AI are consistent, they are small and insufficient to justify administration on a herd basis. Possible explanations for not achieving a larger increase in pregnancies per AI following hCG administration may be that the formation of the accessory corpora lutea and increases in circulating progesterone concentrations are not sufficiently early in the cycle to induce the uterine changes that are needed to ensure embryo survival. Interestingly, the observed increases in circulating progesterone concentrations following hCG administration to lactating dairy cows are still lower than the concentrations observed in normally cyclic heifers (Nascimento *et al.* 2013a).

### Nutrition–energy balance

Following parturition, the nutrient demands of the dairy cow increase markedly as peak lactation yield is approached and typically exceed dietary intake, resulting in a state of negative energy balance (NEB). During this period, body carbohydrate

and protein reserves are mobilised to meet the combined demands of maintenance and lactation. Over the past three decades, intensive genetic selection for milk yield has increased the differences between the potential feed intake and milk yield (for a review, see Drackley and Cardoso 2014). This has resulted in dairy cows that have a greater predisposition for mobilising body reserves and, as a consequence, for NEB. There is evidence that reproductive performance has been decreasing in high-producing dairy cows, especially when animals are under severe NEB (Nebel and McGilliard 1993). It is also apparent that the consequences are greater when high genetic merit dairy cows are grazing (for a review, see Diskin *et al.* 2006). Recent studies (Morris *et al.* 2009; Wathes *et al.* 2009) that examined gene expression in the uterine tissue and spleen of cows with severe NEB (SNEB) post partum indicated that these cows had increased expression of many key genes known to be involved in inflammatory responses consistent with remodelling of the post partum uterus and the clearance of microbial infections. Cows in SNEB had a delayed immune response and the pattern of gene expression in the spleen indicated that part of the reason was that immune cells were exposed to an environment of increased oxidative stress causing a reduction in genes encoding cytokines that are essential for a normal immune response cascade. Therefore, SNEB, which is primarily a function of dry matter intake (DMI) relative to yield, may prevent cows from mounting an effective immune response to the typical microbial challenge experienced after calving, prolonging the time required for uterine recovery and compromising subsequent fertility (LeBlanc 2008). This further emphasises the desirability to avoid excessive NEB in early lactation.

### Energy balance during the early post partum period and subsequent fertility

A greater number of ovulatory oestrous cycles preceding insemination have been shown to beneficially affect subsequent pregnancies per AI. There is evidence from several studies (Beam and Butler 1997; Patton *et al.* 2007) of an association between the energy balance (EB) during the first 3–4 weeks of lactation and the timing of the first post partum ovulation. Consequently, it is desirable that dairy cows resume ovulation in the first 4 weeks after calving. Patton *et al.* (2007) explored the relationships between EB, DMI and peripheral concentrations of insulin-like growth factor (IGF) 1 measured during the first 28 days of lactation and subsequent pregnancies per AI. The first-service pregnancies per AI rate was positively associated with all three variables. Because there may be long-term carryover effects of nutrition–EB on pregnancies per AI rate, this is a particularly interesting observation, but effects on fertilisation and timing of embryo mortality remain to be documented. Britt (1994) hypothesised that follicles exposed to adverse conditions, such as NEB, during their initial stages of growth would have impaired development resulting in the production of inferior quality oocytes and dysfunctional corpora lutea. This hypothesis has gained significant credence as a possible explanation for low fertility associated with NEB, although the underlying biology has not been adequately tested in cows. Recent studies using a heifer model (Parr *et al.* 2015) challenged

the hypothesis that low pregnancy per AI in cows is caused by NEB and its associated metabolic changes occurring in early lactation. Despite significant loss of live weight (LW;  $5.8 \pm 2.1$  vs  $70.5 \pm 2.8$  kg) and BCS ( $0.05 \pm 0.03$  vs  $0.45 \pm 0.03$ ) and a significant increase in systemic concentrations of non-esterified fatty acids in heifers fed a restricted diet for 50 days compared with those fed a normal diet, there was no concurrent effect on pregnancy per AI (69% vs 72%) following AI at Day 50 (Parr *et al.* 2015). However, there was a carryover effect on pregnancy per AI because there was an 18 percentage point difference in favour of the restricted heifers (64% vs 82%) between control and restricted heifers following AI on Day 93. The results of that study indicate that, at least in heifers, a reduction in energy intake for a 50-day period before insemination had no concurrent effect, but did have a positive carryover effect on pregnancy per AI. Therefore, it can be concluded that NEB had no effect on the oocyte's ability to form an embryo and establish a pregnancy. The results should be viewed in the context that the origin of NEB in dietary-restricted heifers is different to that of post partum lactating cows.

Recently, several laboratories have examined the effects of glucogenic–lipogenic feeding strategies to dairy cows on reproductive function (Garnsworthy *et al.* 2009; for a review, see Friggens *et al.* 2010). There is evidence that the feeding of a glucogenic diet that promotes increased circulating insulin and glucose concentrations (van Knegsel *et al.* 2005; Rizos *et al.* 2008) would be expected to improve several reproductive variables. Gong *et al.* (2002) found that the feeding of a glucogenic diet increased circulating concentrations of insulin and increased the proportion of cows ovulating by Day 50 after calving, which is highly desirable. However, there is evidence that high circulating concentrations of insulin have negative effects on oocyte quality (Fouladi-Nashta *et al.* 2005; Garnsworthy *et al.* 2008a) and on *in vitro* embryo production from overfed superovulated heifers (Freret *et al.* 2006). In contrast, lipogenic diets increased the oestradiol secreting capacity of the preovulatory follicle, provided enhanced substrate for progesterone production (Leroy *et al.* 2008) and improved blastocyst development rates. Garnsworthy *et al.* (2008b) have proposed a strategy of glucogenic feeding during the early post partum period to hasten the onset of regular oestrous cycles followed by more lipogenic feeding to lower circulating insulin and improve oocyte quality. Although there is a strong physiological basis for this strategy, there is a need for large-scale confirmation studies.

#### **EB around the time of insemination and subsequent pregnancies per AI**

DMI is lower for cows grazing pastures than for cows fed TMRs. High-genetic merit high-producing cows experience greater NEB in early lactation under grazing conditions relative to lower-genetic merit cows, notwithstanding the somewhat higher DMI capacity of the former cow type (Horan *et al.* 2004). Meta-analysis of the combined data of Kennedy *et al.* (2003) and Horan *et al.* (2004) showed that there was no service  $\times$  study  $\times$  supplementation rate, service  $\times$  study or study  $\times$  supplementation rate interaction effects on pregnancies per AI (see Diskin *et al.* 2006). However, there was a service  $\times$

supplementation rate effect on conception rate. The rate of supplementation had no effect on first-service pregnancies per AI (56% vs 53%), but cows on the low level of supplementation had a lower ( $P < 0.05$ ) second pregnancies per AI rate compared with cows on the high level of supplementation (39% vs 58%). Interestingly, the withdrawal of concentrate supplementation commenced at about the onset of the breeding period in both studies. From these studies, there is no clear evidence that concentrate supplementation of dairy cows at pasture improves first-service conception rate, but it may be beneficial in maintaining second-service conception rates. This clearly highlights the difficulty that improving the EB of the modern dairy cow presents at this stage of lactation where grazed grass is the predominant component of the diet. Based on the studies of Sangsritavong *et al.* (2002), the increased milk production resulting from concentrate supplementation may well be associated with increased hepatic blood flow and increased metabolism of progesterone with the predisposition to greater risk of embryo death.

#### **Sudden reductions in feed intake and pregnancies per AI**

Studies with heifers show that sudden reductions in DMI around the time of AI adversely affect embryo survival, measured by ultrasonography at around Day 30 after AI (Dunne *et al.* 1999). When energy intake was reduced from a high level of twice the maintenance requirement to 0.8-fold maintenance for 2 weeks immediately after AI, pregnancies per AI rates in heifers were consistently less than 40%. When heifers were provided with either a constant level of feed intake or changed from a low to a higher level feed intake, embryo survival was 65%–71% (Dunne *et al.* 1999). In that study in heifers, there was no indication of any association between energy intake and systemic progesterone concentrations. Unlike the situation in sheep and pigs, there was no change in systemic progesterone concentrations following either an increase or reduction in energy intake; changes in progesterone metabolism may have been balanced by changes in progesterone production.

#### **Infectious diseases**

Many bacterial, viral, protozoan and fungal infections have been associated with early embryo, late embryo, fetal and abortion losses, as well as with perinatal losses in cattle (for a review, see Givens and Marley 2008). The outcomes of infectious insults to the bovine conceptus depend on the predilection of the insulting agent for the gravid reproductive tract, the virulence of the insult and the developmental maturity and/or immune competence of the conceptus at the time of the insult (BonDurant 2007). Frequently, the losses occur from mid to late gestation; therefore representing a significant economic loss to the producer and highlighting the importance of control measures to prevent infection.

#### **Practical strategies to enhance embryo survival rates at the farm level**

Although herd health, as a factor affecting pregnancies per AI, is not the focus of this review, it is clear that attention to the health status of individual cows and the herd not only affects

production efficiency, but can also directly or indirectly affect reproductive performance. Consequently, measures to control the health status of individual cows and of the herd must be paramount. Although our understanding of nutrition-reproduction interactions is far from complete, cows should be fed diets that meet their nutrient demands. Specifically, producers should ensure that:

- high DMI and energy intake during lactation is achieved by providing a palatable, energy-dense balanced diet with adequate feeding space and a plentiful supply of clean water
- the BCS of cows is measured on a regular basis to monitor diets and make appropriate adjustments
- cows should be in moderate BCS at calving
- during the breeding season, sudden reductions in dietary intake are avoided
- although the effects of crude protein intake on pregnancies per AI are equivocal, it is best to avoid the feeding of excessive quantities of rumen-degradable crude protein in particular to cows
- significant emphasis is placed on increasing submission rates by improving heat detection efficiency and accuracy, perhaps by using automated heat or ovulation detection technologies and other technological innovations (better heat detection will also improve the timing of AI and reduce the submission of cows that are not in heat for AI; improving submission rates can partially offset the consequences of low embryo survival rates)
- controlled breeding systems that elevate circulating progesterone concentrations in the cycle before insemination may be considered, particularly in environments where both herd conception and heat detections rates are low
- high-quality semen from high-fertility bulls is used
- semen is stored, handled and thawed using correct procedures
- cows are inseminated correctly by placing the semen in the uterine body
- where high ambient temperatures occur, shade, sprinkler or forced ventilation systems are used to mitigate these effects
- particularly in seasonally calving herds, replacement heifers are well grown at the planned start of mating period and are bred in the early part of the breeding period to calve early in the following calving season
- a balanced breeding program with emphasis on both production and fertility traits is adopted when selecting sires to produce herd replacements (breeding companies should ensure that the sires and dams of future AI bulls are not carriers of genes with known adverse effects on cow fertility)
- inbreeding of both the cow and the potential embryo is minimised by the use of computer-based mating programs that allow producers to maximise specific breeding objectives while constraining inbreeding of the expected progeny.

Cross-breeding, which can increase reproductive performance, is an option in some production systems and results in immediate improvement in fertility in the  $F_1$  progeny. Finally, it should be kept in mind that the cumulative effects of correcting minor deficits in several areas can have a substantial overall effect.

## Future possibilities

It is well established that embryo survival is critically important in cattle, and particularly in seasonally bred herds. The extent of late embryo mortality is numerically much smaller than early embryo mortality but nevertheless causes serious economic losses to producers because it is often too late to rebreed females when they repeat in all herds, and particularly in seasonally bred herds. The major challenge will be to improve pregnancies per AI and therefore herd reproductive efficiency, particularly given the antagonistic relationship between milk production and embryo survival rate. It is clear that genetic variability exists within the Holstein breed for important fertility, DMI and EB traits (Berry *et al.* 2007). Because of the difficulty in directly measuring DMI and/or EB in large numbers of dairy cows, it is not currently directly included in international breeding objectives (Berry *et al.* 2014b). Applying a positive weighting to traits correlated with DMI and/or EB, or the identification and application of suitable molecular markers, would be expected to improve both, as well as subsequent reproductive performance (Pryce *et al.* 2014).

Alternatively, strains of cows derived from more balanced breeding objectives, such as those practiced in New Zealand (Harris and Kolver 2001) and France (Barbat *et al.* 2010), or alternative dairy breeds, such as the Jersey, Norwegian Red and Swedish Red breeds, or cross-bred dairy cows could be used particularly in grass-based production systems. In a recent review, Buckley *et al.* (2014) clearly illustrated favourable reproductive performance benefits from cross-breeding (e.g. first-service pregnancies per AI increases of between 8 and 21 percentage points) and cow longevity using a range of modern breeds within both grass-based and high-input confinement production environments. Cross-breeding, as opposed to longer-term genetic improvement strategies, provides and immediate improvement in fertility in the  $F_1$  progeny and can be viewed as a relatively rapid means of improving herd reproductive performance.

Advances in genomic technology have the potential not only to provide scientists with an improved understanding of the underlying biological processes involved in fertilisation and the establishment of pregnancy, but also to explore gene profiles and potentially identify genes responsible for improved embryo survival. Genomically assisted selection will increase the rate of genetic progress for reproductive traits such as embryo survival by increasing the accuracy of selection and permitting selection in both genders at a young age. High-throughput SNP genotyping and whole-genome sequencing combined with large databases and good phenotypic data now afford unique opportunities to gain new insights not only into the detection of genetic defects, but also into the underlying genetics of embryo development and cow fertility (Fortes *et al.* 2013). Existing well-established reproduction-enhancing technologies such as AI, embryo transfer and ovum pick-up will have an important role in fully exploiting their potential.

Once cows have recommenced regular oestrous cycles after calving, subsequent herd reproductive performance is the product of submission rate (heat detection efficiency) by pregnancies per AI (Diskin 2008). Increasing the heat detection rate from

50% to 70% will have the equivalent effect on overall herd reproductive performance as increasing herd pregnancies per AI by 13–15 percentage points (Diskin 2008). It is difficult to provide practical recommendations that repeatedly ensure high embryo survival rates in contrast with firm recommendations that can reliably and consistently improve submission or heat detection rates. Consequently, it is advised that producers should concentrate on improving submission rates by improving heat detection practices. Furthermore, heat detection practices, although time consuming and repetitive, are largely under the control of herd managers. Improvements in more automated heat detection technologies (for a review, see Fricke *et al.* 2014) will also improve submission rates. Further large field-scale studies are required to definitively establish the herd circumstances that would benefit from direct progesterone supplementation or from increasing endogenous output of progesterone by hCG administration. Similarly, it is currently premature, in advance of confirmation from comprehensive feeding studies, to advocate dietary manipulation (glucogenic and or lipogenic) to enhance embryo survival rates.

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