An International Conference

Fertility in Dairy Cows

bridging the gaps

30-31 August 2007

Liverpool Hope University, UK

organised by the

British Society of Animal Science

and

University of Liverpool

EAAP satellite meeting

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Foreword

Dairy Cattle are an integral part of agriculture worldwide, providing many products in addition to milk for the human population. The efficient production of these products is of utmost importance and high reproductive performance is absolutely crucial to this. In September 1999, *Dr Michael Diskin* led a committee (*Dr Joseph M. Sreenan, Prof James Roche, Prof Maurice Boland and Dr Diarmuid O'Callaghan*) to organize an extremely successful and informative occasional meeting jointly with the British Society of Animal Science in Galway, Ireland to address the important issue of declining reproductive performance in dairy cattle. The title of the meeting was "Fertility in the High-Producing Dairy Cow". As discussed by *Dr John Robinson* in the conference summary, "….delegates after a starting point of uncertainty about how to deal with the problem, came away very well informed scientifically and, if perhaps with varying views as to the best way forward, more aware of the multi-disciplinary research and development approach now being used to investigate it". Dr Robinson concluded his summary by stating "Reversing this relentless decline in dairy cow fertility, while simultaneously sustaining high yields, is not going to be easy……it is a challenge that will require great interchange of information and ideas between science, practice, research and development. The establishment of contacts and beginning of such interchange has been initiated during this [1999; Galway] meeting and it is essential that this is sustained."

Now, exactly 8 years later it is time for those representatives from all disciplines involved in attempting to improve dairy cattle fertility to reconvene. Since 1999, the continued and well-documented decline in dairy cow fertility has prompted new research into diverse aspects of dairy cow reproduction. It has also prompted the development of new technology and applications in the field. The purpose of this conference is to provide an update on these activities since 1999 and to provide a forum for bridging the gaps between the different disciplines involved.

Invited and submitted theatre and poster sessions include new, up and coming presenters in addition to keynote speakers from across the globe, recognized as international experts in their field. As was the case at the 1999 meeting, the content is of direct interest to scientists, university lecturers, veterinarians, farm advisors and technical representatives working within the dairy industry as well as many dairy farmers and will include sessions dealing with the following topics:

- Pre-disposing factors for fertility
- New opportunities in fertility management
- Better indicators of reproductive ability
- Impact of new on-farm technologies on reproductive management

Finally, via this meeting we hope to promote one of the major aims of the British Society of Animal Science which is to encourage fruitful exchange of information and ideas between all of those involved in the science and practice of animal production.

The organizers are very grateful for the sponsorship from BASF, Genesis Faraday, Genus ABS, Holstein UK and CIS, Merial, Pfizer, Reproduction Specialities Inc, The Stapledon Memorial Trust and World Wide Sires Ltd. We would also like to thank everyone who has contributed to bringing this excellent international conference to fruition.

Organisers
Dr Melissa Royal (University of Liverpool, UK)
Dr Nic Friggens (University of Aarhus, Denmark)
Dr Robert Smith (University of Liverpool, UK)
Mr Mike Steele (British Society of Animal Science)

ISBN 0 906562 59 7

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Thursday 30 August

Friday 31 August

08:45 SESSION 3: *(session supported by BASF)*
New Opportunities in Fertility Management
Chair - Lucy Andrews

14:00 SESSION 4: *(session supported by Holstein UK and CIS)*
Better Indicators of Reproductive Ability
(are we collecting the right data and are we using it properly?)
Chair - Mike Coffey

Posters

Reproductive challenges facings today's dairy industry

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It has been well documented that fertility in the UK dairy industry has been declining over the past 20 years (Royal et al 2000a) simultaneous to that observed worldwide. However, this phenomenon is a multi-factorial problem involving many aspects of the dairy industry which has altered and evolved in the last several decades. The economic demand for more profitable dairy enterprises has been the driving force behind increased genetic selection pressure on production which indirectly has had an unfavourable impact on fertility both at a phenotypic and genetic level (Royal *et al*. 2002a,b). However, since fertility is a quantitative trait we must be clear that yield and hence indirect genetic selection against fertility is not the only reason for the decline. Increased herd sizes, changing farming systems, less skilled labour, disease and our ability to manage, feed and select dairy cows with desirable reproductive traits have all impacted on the ability of the dairy cow to rebreed.

The ultimate objective with regards to rebreeding in reality is extremely demanding, since what we actually desire is for a cow to conceive at a desired time after calving, to establish and maintain a pregnancy in a designated set period of time and so, produce a viable calf at a preferred date in the future. In order for a cow to accomplish this, synchrony between a number of physiological and managerial processes must be achieved (Darwash *et al*., 1999). These include commencement of luteal activity; visible manifestation of oestrus; timely AI by a skilled technician using fertile semen; the shedding of an oocyte capable of being fertilized and the secretion of adequate hormone levels essential for optimal tubal and uterine environments to promote successful pregnancy and maintenance of the developing embryo. However, when asked directly what today's main fertility concerns are, dairy farmers frequently respond "getting cows back in calf." This apparently simple and rather banal response is thus the common symptom arising from one of many possible causes thus highlighting the immensely complicated and multi-factorial nature of the problem we all face as farmers, scientists, veterinary surgeons and breeders.

This paper wishes to begin this meeting by highlighting the reproductive challenges facing today's dairy industry particularly in today's current economic climate. Moreover, it aims to highlight the perceptions, expectations and problems of dairy cow fertility at the farm level for both the UK dairy farmer and cattle veterinary surgeon. Are the opinions of the dairy farmer and veterinary profession in synch with those of the scientific community? If so, exactly how are these perceptions, expectations and problems being tackled by research scientists worldwide and to what extent are the fruits of such research projects being communicated and implemented in the dairy industry? If not, is it time to reassess exactly what the dairy farmer is looking for to improve fertility at the farm level.

The relationship between fertility and farm economics is well established (Esslemont 1982; Stott *et al*., 1999) and continuing investment in fertility research is dependant on continual improvement in farm output. It is reasonable that applied research must retain a clear vision of how research findings can be translated into changes and improvements in fertility performance at farm level. New tools and techniques to assist in the delivery of researched solutions could help ensure the survival and progression of the UK dairy industry.

The real challenge facing today's dairy industry is not just in learning more about the cows and their environment. It is also in researching how farmers interact with their farm and their livestock. The obstacles to "change" are very real for farmers yet there are few sectors of the UK population with a stronger work ethic or commitment to what they do each day. For vets and researchers alike, effecting permanent management change can be extremely challenging. It is perhaps time to refocus research sights on the areas that can deliver the maximum farm level benefits through the smallest changes in farm routines.

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The concept of developmental programming of reproduction in farm animals

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The phenotype of an individual is now widely regarded as being ‘plastic’ or susceptible to changes in its environment early in development. Such plasticity confers phenotypic adaptability in the face of a changing environment and is thus beneficial on an evolutionary timescale. As a result of the agricultural and industrial revolutions, the environment in which many animals now live has changed rapidly; too quickly for their ‘paleolithic’ physiology to cope and increased ‘lifestyle-associated’ diseases are prevalent. Professor David Barker was the first to expound the concept of developmental programming, in the context of the increasing prevalence of non-communicable disease, in the late 1980’s. Here an adverse early environment (marked in many early studies by reduced birth weight) impacts upon an individuals physiological and metabolic competence in middle age (e.g. to increase the risk of hypertension, obesity, Type 2 Diabetes). Many animal studies that are free from the multivariate confounding endemic with human epidemiological work have now illustrated the biological plausibility of the hypothesis and have extended the general framework to virtually all aspects of physiological/metabolic function. Interestingly, the effect (metabolic programming) has also been shown to pass through generations, in both human epidemiology and animal studies; a modern synthesis of the works of both Darwin and Lamarck. Such a remarkable effect implies that developmental programming influences gonadal development, reproductive function and thus fertility. Indeed, there is some evidence to suggest that the plane of maternal nutrition can impact upon ruminant reproductive function and fertility (Robinson *et al.*, 2006). The extent to which such developmentally programmed sequelæ are transmitted predominantly down the male or female line has yet to convincingly explored. This paper will consider whether these effects may be attributable to direct effects of maternal undernutrition on fetal/postnatal gonadal development or, at least partly, occur as a secondary consequence of alterations to maternal hormonal status. The communication will incorporate the results of recent work in both sheep and cattle.

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Factors influencing heifer survival and fertility on commercial dairy farms

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Fertility in dairy cows has declined steadily over the past 40 years. The average UK cow survives only 3 lactations, with infertility the major cause for culling. The availability of heifers to enter the herd is therefore reducing at the same time that demand for them is increasing. We have investigated both the timing of heifer deaths and possible contributing factors. Many potential heifers are lost pre-natally due to early embryonic death (about 40%), later embryo loss (at 1-2 months, 20%) and abortion (about 5%). Our recent survey of 19 UK herds showed that 8.1% of calves were born dead and a further 3.4% died within their first month. Of 434 heifer calves recruited onto a study at 1 month of age from these herds, 27 (6.2%) had died or been culled before reaching their first service period at 15 months due to either disease or accident. Some heifers served at this stage failed to conceive (14/407, 3.4%). Of 363 heifers that have reached first calving, 12 (3.3%) were removed from the herd within 8 weeks, predominantly as a result of difficult calvings and mastitis. There is therefore steady attrition of potential replacement heifers before they even enter the herd; such losses are generally not accounted for on the farm.

Once the heifers do enter the milking herd, the most profitable animals are those which manage to combine good milk production qualities with a regular calving pattern. We have investigated some possible juvenile predictors of future performance. Low birthweight calves (32 ± 0.5 kg) were more likely to come from older dams (3+ lactations) with higher peak milk yields (>42 kg/day) in comparison with high birthweight (42 ± 0.8 kg) calves. Sire affected gestation length but not birthweight. This suggested that the maternal uterine environment may limit pre-natal calf growth due to competition for nutrients with those going into milk production. Subsequent milk production was not, however, affected by birthweight. Postnatally, sire showed a greater influence with significant estimates for sire heritability on weight, body condition score, IGF-I, insulin and milk production parameters in the first lactation, coupled with a significant effect on conception rates at this stage.

As both milk production and fertility are strongly regulated by the somatotrophic axis, we determined whether measures of somatotrophic hormones at 6 months of age could predict actual performance over 3 lactations. Neither endogenous nor stimulated GH release patterns were related to peak or 305 day yield in any lactation, although the size of a stimulated GH peak was positively related to milk energy values in the first lactation. Pre-pubertal IGF-I and insulin concentrations also failed to predict milk production. Cows with delayed ovulation in the first lactation (>45 days to first progesterone rise) did, however, have a higher GH pulse amplitude and lower IGF-I as a juvenile.

Age at first calving (AFC) is another parameter known to influence later performance. AFC can be influenced by heifer growth rates and management decisions on when to serve. Heifers not calving until >25 months required significantly more services/conception than those calving at a younger age. Calving at over 30 months of age was associated with severe problems over the peripartum period. Optimum fertility and maximum yield in the first lactation were associated with an AFC of 24-25 months. However, heifers calving at 22-23 months performed best in terms of total milk yield and survival in the herd over the first 5 years of life. This was in part because good fertility as a heifer was associated with better fertility later in life as a cow.

In summary, many potential replacement heifers never enter the herd due to disease, accident or poor fertility. This severely limits any opportunities for on-farm selection. Good fertility as a heifer predicts good performance later. Conversely, heifers which experience initial difficulty in conceiving calve later and then perform badly.

We thank the Milk Development Council and Defra for supporting this work.

Why is it getting more difficult to successfully AI dairy cows?

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Successfully using AI is defined as getting cows pregnant when the farmer wants them in-calf and making best use of appropriate genetic potential. The percentage of oestrous animals standing-to-be-mounted (STBM) has declined from 80% to 50% over the past 30-50 years and the duration of STBM from 15h to 5h; both in parallel with a reduction in first-service-pregnancy-rate from 70% to 40%. Meanwhile, the incidence of lameness has increased; and it takes more than an extra 40 days to get a lame cow in-calf compared to healthy herd-mates. The intensity of oestrus is 50% lower in severely lame cows and fewer lame cows ovulate. Milk progesterone concentrations are also 50% lower in lame cows, and oestradiol is also lower in non-ovulating lame cows compared to ovulating animals. Furthermore, cows that do not ovulate do not have an LH surge and LH pulse frequency in their late follicular phase is lower; 0.53 versus 0.76 pulses/h. Thus, we suggest that the stress of lameness reduces LH pulsatility required to drive oestradiol production by the dominant follicle. The consequent low oestradiol results in less intense oestrus behaviour and failure to initiate an LH surge; hence there is no ovulation. A series of experimental studies substantiate our hypothesis that events activating the hypothalamus-pituitary-adrenal axis interfere at both hypothalamus and pituitary level to disrupt LH and oestradiol secretion, and thus expression of oestrus behaviour. Our inability to keep stress at a minimum by appropriately feeding and housing high-production cows is leading a failure to meet genetic potential for yield and fertility. We must provide realistic solutions soon, if we want to successfully use AI to maintain a sustainable dairy industry for the future.

Metabolic changes in high yielding dairy cows and the consequences on oocyte and embryo quality

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Disappointing reproductive performance, such as reduced conception rates but also an increased incidence of early embryonic mortality, is reported world-widely and has been associated with the period of negative energy balance (NEB) early post partum. This NEB is featured by typical biochemical changes such as high non-esterified fatty acid (NEFA), high β-hydroxybutyrate (BHB) and low glucose concentrations. Besides many others, the concentrations of these metabolites were extensively analysed in the follicular fluid (FF) of high yielding dairy cows during NEB and were imitated in *in vitro* maturation models to investigate their effect on oocyte quality. Serum free maturation media (20ng/ml mEGF) were supplemented with the three predominant free fatty acids (oleic, stearic and palmitic acid) at concentrations that were found in the FF. Nuclear maturation rate, fertilisation and cleavage rates were assessed as well as the embryo yields. Also the effects of very low glucose and high BHB concentrations during oocyte maturation, imitating the FF concentrations during ketosis, were tested. The results showed that the typical metabolic changes during NEB are well reflected in the follicular fluid (FF) of the dominant follicle. However, the oocyte seems to be relatively isolated from too high NEFA or too low glucose concentrations in the blood. Nevertheless, the in vitro maturation models revealed that such NEB associated high NEFA and low glucose concentrations in the FF are indeed toxic for the oocyte, resulting in a hampered oocyte maturation and developmental competence. This was especially seen in the induced apoptosis and necrosis in the cumulus cells. BHB had an additive toxic effect but only in moderately hypoglycaemic maturation conditions. These in vitro maturation models, based on in vivo observations, suggest that a period of NEB may hamper fertility of high yielding dairy cows through increased NEFA and decreased glucose concentrations in the FF directly affecting oocyte quality.
Apart from oocyte quality, we also found that embryo quality is reduced even when the episode of NEB is passed. This important observation may be linked to the typical milk yield stimulating diet or to physiological adaptations sustaining the high milk production. Research is ongoing to study this phenomenon.

Effects of progesterone on embryo survival

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Successful genetic selection programmes over the past 30-40 years have resulted in a modern dairy cow, biologically efficient at producing large volumes of milk but with a significantly decreased fertility level. Currently the average embryonic and foetal loss rate in high producing dairy cows is close to 60%. It has been established that 70-80% of this total loss occurs during early embryo growth, i.e. between days 8 and 16 after insemination (Diskin and Sreenan, 1980). The data currently available on the extent and timing of early embryo loss and on aspects of embryo growth, development, metabolism and viability in cattle show that during the period of greatest embryo loss there are dramatic changes in embryo growth with an exponential increase in embryo size and protein content. At this time embryos are undergoing time and developmental stage dependent changes in the rate of *de novo* protein synthesis and protein phosphorylation (Morris *et al*., 2000).

Progesterone has been called the 'hormone of reproduction' and inadequate systemic concentration of progesterone during the early luteal phase is likely to be responsible for a proportion of this embryo loss. We have recently shown, in a relatively large study, a linear and quadratic relationship between the concentration of progesterone in the first few days after AI and embryo survival rate. In that study low concentrations of progesterone on day 5 to 7 or a low rate of increase in progesterone between days 4 to 7 was associated with a low probability of embryo survival (Stronge et al, 2005). Supplementation of animals at risk with progesterone has been shown to reverse this loss and supplementation has been associated with a more developed embryo capable of producing increased quantities of interferon tau by day 16. Until the embryo implants at around Day 20 normal embryo development is dependent on an adequate uterine supply of nutrients and growth factors. The uterus, however, is dynamic and capable of maintaining a pH lower than blood (Hugentobler *et al*., 2004) while at the same time maintaining uterine fluid concentrations of ions, amino acids and energy metabolites that are either lower or higher than that of blood and vary with stage of the oestrous cycle. This dynamism, however, makes it susceptible to external influence such as that posed by changes in the concentration of systemic progesterone. The molecular mechanism, however, through which progesterone affects uterine function and subsequent embryo survival remains poorly understood. A recent study by McNeill *et al*., (2006) has shown that steroid responsive genes, regulated by the interplay of estrogen and progesterone with their respective receptors are sensitive to small changes in the concentrations of systemic progesterone particularly in the early luteal phase of the oestrous cycle when progesterone per se is low. One of these genes, encoding retinol binding protein, which is essential for the transport of Vitamin A, shows stage of cycle and concentration dependent changes in its expression. Current studies from this laboratory are attempting to further elucidate the mechanisms affecting embryo loss by examining the uterine proteome and determining how it is affected by changes in the systemic concentration of progesterone and by stage of cycle.

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Effect of Escherichia coli infection of the bovine uterus from the whole animal to cell

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Following parturition, contamination of the uterine lumen by bacteria is ubiquitous, and uterine health is impaired in cattle because infection persists in 10 – 15 % of animals as endometritis. Endometritis causes infertility for the duration of infection, however, subfertility persists even after successful resolution of the disease. *Escherichia coli* (*E. coli*) is the pathogenic bacterium most frequently isolated from the bovine postpartum uterus, and its isolation is associated with increased peripheral acute phase protein concentrations and fetid vaginal mucus. The presence of *E. coli* is also associated with a slower growth rate and lower estradiol secretion of the first postpartum dominant follicle. Furthermore, in animals that ovulate the first dominant follicle, the corpus luteum formed is smaller and secretes less progesterone.

Absorption of bacterial components from the uterus can prevent the follicular phase luteinising hormone (LH) surge and ovulation. The endotoxin lipopolysaccharide (LPS), which is released from *E.coli*, can pass from the uterine lumen to the peripheral circulation and concentrations are increased in cows with uterine infection. Infusion of *E. coli* LPS into the uterine lumen suppresses the pre-ovulatory LH surge and stops ovulation in Heifers. *In vitro*, endometrial explants produce prostaglandins in response to LPS. Addition of LPS or *E. coli* to stromal and epithelial cells stimulates the production of PGE and PGF and increases their cyclooxygenase 2 mRNA expression. Furthermore, these cells express mRNA for the molecules required for recognition of LPS, Toll-like receptor-4 and CD14.

In summary, *E. coli* is recognised *in utero* and this results in the modulation of the endocrine function of uterine cells and, in addition to the direct effects on the uterus, the subfertility associated with *E. coli* infection involves perturbation of the hypothalamus, pituitary and ovary.

Progesterone profiles and embryo development in beef heifers with elevated progesterone concentrations

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Introduction Elevated concentrations of circulating progesterone in the immediate post-conception period have been associated with an increase in embryonic growth rate, interferon-tau production and pregnancy rate in cattle (Garrett et al., 1988; Mann and Lamming 2001). Much of this effect is likely mediated via downstream effects of progesterone-induced changes in gene expression in the tissues of the uterus (Satterfield et al., 2006). However, whether or not progesterone has a direct effect on the embryo also is unknown and, at least in vivo, is difficult to assess. The objective of this study was to examine the influence of circulating progesterone concentrations on embryo development on Days 5, 7, 13 and 16 post-conception.

Materials and methods Beef cross heifers were synchronized using a CIDR device for 8 days with a prostaglandin $F_{2\alpha}$ analogue administered 3 days before CIDR removal. Only animals exhibiting a clear standing oestrus (n=210), defined as Day 0, were used. In order to produce animals with divergent progesterone concentrations, half of the animals received a PRID on Day 3 of the oestrous cycle which was left in place until slaughter. The 4 treatment groups were (i) Pregnant, high progesterone, (ii) Pregnant, low progesterone, (iii) Non-inseminated, high progesterone, and (iv) Non-inseminated, low progesterone. All animals were blood sampled twice daily from Days 0 to 8 and once daily thereafter until slaughter. Animals were slaughtered at Day 5, 7, 13 and 16 corresponding to the 16-cell stage, the blastocyst stage, the beginning of elongation and the day of maternal recognition of pregnancy, respectively. Embryos were recovered by flushing the tract and characterized by stage of development and, in the case of Days 13 and 16, measured. Data were analyzed by Chi-square analysis and Student's t test where appropriate.

Results Insertion of a PRID on Day 3 resulted in a significant elevation in progesterone concentrations from Day 3.5 onwards (Figure 1). There was no difference in the proportion of embryos at the expected stage of development between Days 5 or 7 (P>0.05). However, elevation of progesterone resulted in a significant increase in embryo length on Day 13 (2.24±0.51 vs 1.15±0.16 mm, P=0.034) and Day 16 (14.06±1.18 vs 5.97±1.18 cm, P=0.012).

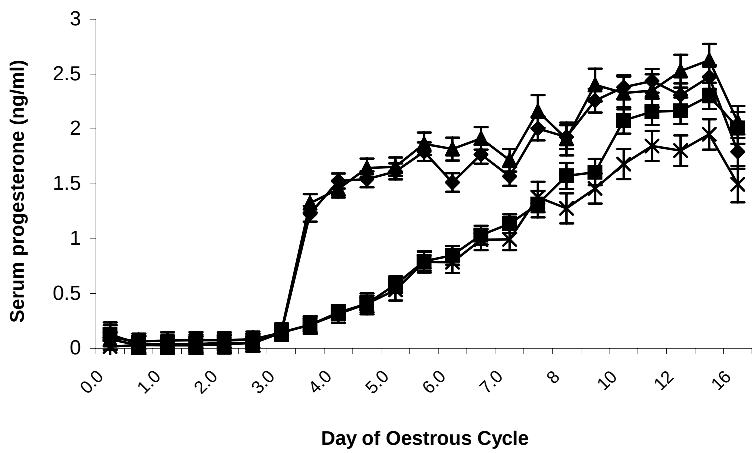

Figure 1 Serum progesterone concentration (mean±s.e.) in pregnant (■,♦) and non-inseminated (▲,x) beef heifers treated (♦,▲) or not (■,x) with a PRID device from Day 3 until slaughter at either Day 5, 7, 13 or 16.

Conclusion Insertion of a PRID on Day 3 of the oestrous cycle results in a significant rise in serum progesterone concentrations on subsequent days which is associated with an increase in embryo dimensions on Days 13 and 16.

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Milk progesterone as a genetic and management tool to improve fertility in dairy cows

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Introduction Studies of the interval from calving to commencement of luteal activity (CLA; occurring about four to five days after first ovulation) have revealed heritabilities of 16 to 21%, which is considerably higher than for the present measures of fertility in dairy cows (e.g. Veerkamp *et al*., 2000; Royal *et al*., 2002). In these studies of CLA, progesterone samples were taken relatively frequently. Such frequent sampling would require in-line progesterone monitoring systems. A suggested alternative is to use milk samples from the routine milk recording for analysis of progesterone (van der Lende *et al*., 2004). The disadvantage is that milk samples in the regular milk recording are taken relatively infrequently (once every 4 – 6 weeks), and it needs to be determined how sampling intervals affect the genetic parameters of progesterone-based measurements. From a management point of view, continuous information about the probability of normal ovarian cyclicity during the postpartum period based on progesterone profiles would indeed help both the herdsman and the veterinarian to identify abnormal cows and to administer the most appropriate reproductive treatment. This consequently increases the probability of achieving reproductive goals. The aims of this study were to investigate how milk progesterone measures based on different sampling intervals could be used in breeding programmes to select for an earlier first ovulation after calving, and as a diagnostic tool at an individual level for disturbed ovarian function.

Material and methods Genetic analyses were conducted using a milk progesterone database from the University of Nottingham, UK and Roslin Institute, UK with additional information from two commercial databases from National Milk Records Plc, Chippenham, UK and Holstein United Kingdom, Ricksmanworth, UK. These data were collected between October 1996 and March 1999 and comprised 1212 lactations from 1080 British Holstein cows in eight herds. A mixed linear animal model was fitted to the data and variance components were estimated using REML. The management study was made using data from a Swedish experimental herd and collected between December 1987 and December 2002. In this data, a total of 1040 lactations were included from 507 dairy cows of Swedish Red (n = 324 cows) and Swedish Holstein (n = 183 cows) breeds. The full dataset was divided randomly into one test set (n = 550 lactations) and one validation set (n = 490 lactations). The British dataset presented above was also used for an additional validation. A logistic regression was fitted to the test data, with delayed cyclicity or not as the dependent variable. The basic model included effects of breed, parity, season and housing. To this model we then added a fixed regression on various progesterone based measurements. The various progesterone measures were CLA (transformed for genetic analyses with natural logarithm, lnCLA) and percentage of samples within the first 60 days postpartum above the minimum milk progesterone concentration for luteal activity (PLA) both based on actual milk sampling for progesterone two to three times per week, and CLA and PLA based on monthly sampling within the first 60 days postpartum (CLA_m and PLA_m).

Results The mean heritability estimate was 0.09 for CLA_m and 0.11 for PLA_m. Heritability estimates for lnCLA (0.16; Royal *et al*., 2002) and PLA (0.30; Petersson *et al*., 2007) have been reported previously. The genetic correlations between PLA_m or CLA_m and lnCLA had on average an absolute value of 0.85. Selection index calculations showed that monthly progesterone sampling could be used with high accuracy (0.64-0.72 with 50 daughters per bull) to predict breeding values for CLA. The accuracy of models for detection of delayed ovarian cyclicity was highest (0.94 to 0.99), as expected, when CLA or PLA was included in the model. The accuracy was also significantly higher when CLA_m or PLA_m (0.85 to 0.88) was added to the model compared with the model with no progesterone measure (0.76 and 0.67). The overall percentage of correctly diagnosed lactations, for selected probability cut-offs, were 88 to 98% for CLA or PLA, 80 to 82% for CLA_m or PLA_m and 65 or 68% for the model with no addition of progesterone measures.

Conclusions Progesterone analysis in monthly collected milk samples, resembling the regular milk recording system, could be used to increase the accuracy of a breeding programme for fertility by selecting for an earlier start of cyclical ovarian activity after calving. Moreover, it could be used to predict cows with delayed cyclicity with high accuracy within 60 days after calving. This information could be used for veterinary treatment decisions for individual cows and as a herd diagnostic tool for preventive actions to avoid a delayed start of the service period.

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Does exposure to oestrous vaginal discharge and urine from cows and bulls affect the heart rate in heifers?

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Deteriorating reproduction performance is a growing concern to the dairy industry. Oestrous synchronization through the use of exogenous hormones and timed AI are methods that in several countries have been regarded as a prerequisite for an efficient use of artificial insemination. However, the consumers show increasing reluctance to accept such use of hormones which puts focus on the need for alternative aids in reproduction management.

Studies performed on animals and humans have shown that it is possible to manipulate the length of the oestrous cycle through exposure to pheromones. Few experiments have been conducted on cattle, but they indicate that the bovine oestrous cycle also is susceptible to pheromones. Therefore an extensive study has recently started in Sweden, which aims to investigate the effect of substances thought to contain pheromones on oestrous cycle characteristics monitored by assay of reproductive hormones and ovarian ultrasonography. Since studies based on the bovine oestrous cycle are time consuming and expensive, there is need for a bioassay to evaluate the bioactivity of different substances. In certain insects, pheromones have been shown to induce a changed cardiac activity. In a sub study to the project we wanted to examine whether there were any indications that the same applied to cattle.

Two heifers of the Swedish red and white breed were monitored during three consecutive oestrous cycles including manual oestrus detection and ultrasound scan of the ovaries. The animals were exposed to urine from bulls and to vaginal mucus and urine from cows and water as a control for ten minute periods separated by 10 minutes with no substance. The substances were exposed separately in cassettes attached to a nose ring. The exposures took place once during each oestrus and once between day 9 and 14 in the following cycles. During the exposures the heart rate was registered at five seconds intervals, using a commercial, non-invasive heart rate monitor.

We found no consistent change in heart rate during exposure to any of the substances thought to contain pheromones, neither during oestrus nor during dioestrus. However, we registered a significantly ($p<0{,}001$) higher heart rate in general during oestrus (75.7 beats/min) than during dioestrus (66.3 beats/min). We also noticed a tendency towards synchronization of the oestrous cycles in the two heifers.

To conclude, monitoring the changes in heart rate seems to be a poor bioassay for the evaluation of substances presumed to contain pheromones in cattle. However, a continuous monitoring of the heart rate in heifers could possibly be used as a complement to traditional oestrus detection and more extensive studies in that area would therefore be of interest. The synchronization of the oestrous cycles in the two heifers may have been caused by pheromones. This is encouraging for our further experiments on the effect of pheromones on the bovine oestrous cycle.

The effect of dietary protein content on the expression and detection of oestrus in high yielding dairy cows

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Introduction Poor reproductive performance is a major problem when considering the sustainability of the modern high yielding dairy cow. One of the main contributing factors is the decline in the accuracy of oestrus detection. This decline may result from: insufficient time being allocated to the observation of cows; a reduced expression of oestrus behaviour in the modern high yielding dairy cow (particularly standing immobile on being mounted); or an extended calving period (Mayne, 2006). Standing immobile on mounting is the primary and most dependable sign of oestrus and the best indicator of the cow's pre-ovulatory state. However, the expression of this behaviour has been reported to be as low as 37% when cows are in oestrus (Van Eerdenburg *et al*., 1996). Additional factors affecting the expression of oestrus behaviour are: housing; nutritional status; age and physiological state (Orihuela, 2000).

Materials and methods Ninety Holstein Friesian cows (45 first lactation and 45 between 2nd and 6th parity (mean parity of 3.1)) were allocated to one of three treatments: a high (18%); medium (15%); or low (12%) dietary protein content. Treatment commenced following parturition and all diets were offered as a total mixed ration through electronic feeding gates. Treatments were balanced for calving date, parity, previous lactation yield (rearing treatment for heifers), condition score and live weight. The concentrate to forage ratio was 0.55:0.45 (DM basis) for all diets. The forage component of the diet was 0.60 grass silage and 0.40 maize silage (DM basis), providing 13.1 MJ/kg DM. All animals were housed in a single group with individual feed intakes being recorded. Oestrus behaviours were recorded for a 30 minute period every 12 hours from calving until all animals had reached 140 days post-partum. During the observational period a trained observer walked through the group examining cows for activity. All cows carried plastic identification collars and were freeze branded, allowing ease of identification. Behavioural activities were recorded according to a scoring system developed by Van Eerdenburg *et al*. (1996), with nine key oestrus behavioural activities; each allocated a given number of points. Once the observational period was complete the total number of points was calculated. If the total exceeded 50 points during single or consecutive observational periods the cow was deemed to be in oestrus. Milk progesterone concentrations were used to verify ovulation and were measured twice weekly on Tuesday and Friday (AM milk samples). Data were analysed using the Residual Maximum Likelihood (REML) procedure in GenStat.

Results In this experiment a total of 238 heats were observed (oestrus score >50 points), with 51.7% of these heats being characterised as standing immobile on being mounted. The most frequent behavioural activities displayed were sniffing the vagina of another cow (86.7% of heats) and chin resting (89.5% of heats). There were no direct effects of protein on oestrus behavioural activities in this experiment. Three significant stage of lactation by protein treatment interactions were observed for the behavioural activities: mucus discharge ($P<0.01$), chin resting ($P<0.01$) and mounting the head side of a cow ($P=0.05$). However there were no apparent trends from the predicted means. There was a significant influence of parity on the frequency of mucous vaginal discharge ($P<0.05$), sniffing the vagina of another cow ($P<0.05$), chin resting ($P<0.01$), mounting head of other cow ($P<0.01$) and total number of behaviour activities displayed per heat ($P<0.05$). In all these cases cows displayed fewer behavioural activities than heifers. An increase in the size of the sexually active group (cows in oestrus at the same time, up to 5 cows) significantly increased the expression of sniffing the vagina of another cow ($P<0.05$), mounting or attempting to mount another cow ($P<0.05$), the expression of standing immobile on being mounted ($P<0.001$), the total oestrous score obtained ($P<0.001$) and the proportion of cyclic cows that were diagnosed as being on heat ($P<0.01$).

Conclusions With a relatively low expression of standing immobile on being mounted, the most relevant secondary oestrus behaviours for identifying cows in oestrus were sniffing the vagina of another cow and chin resting. The intensity of the displayed heat is highly variable among individuals and is greatly influenced by number of cows that are in heat simultaneously. Small groups of sexually active cows, which is normal in all year round calving systems, will reduce the number of simultaneous heats, and subsequently the probability of oestrus detection.

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Voluntary waiting period – do dairy farmers make an active choice?

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Introduction Voluntary waiting period (VWP) is the time between calving and the day the farmer chooses to start to breed the cows. This period has in Swedish herds traditionally been around 60 days. Lately, some herds may have adopted a strategy to alter this period for groups of cows or individuals depending on postpartum health, milk yield or parity. There have been reports (Arbel *et al*, 2001) indicating that it is economically better to prolong the calving intervals by prolonging the VWP. This is something that some farmers may approve to and may try to implement at the farm level. DeJarnette *et al*.(2007) reported that 64% of their investigated American herds alter their VWP for one or more reasons. In Sweden, the average interval from calving to first service has continuously increased during the last 20 years. One reason behind this could be that farmers today do not regard 60 days VWP as optimal for all cows in the herd but make differentiated choices for groups of cows. VWP affects common measurements of herd reproductive efficiency, such as average time from calving to first AI and average calving interval, and it is therefore useful to know the VWP of a farm and if the farmer modifies it according to cow characteristics. The purpose of this study was therefore to investigate how Swedish farmers think and what they do regarding to reproductive performance, and in particular if they have a strategy for VWP.

Material and methods A survey was sent by mail to 472 dairy herds in March 2006 along with a letter from the authors and a paid reply envelope. A reminder was mailed, if there was no reply within two weeks, to encourage non-responders to participate. The targeted dairy herds represented a random sample of all herds that had more than 45 milking cows and were registered in the Swedish Official Milk Recordings Scheme (SOMRS), in total 2,728 herds. In total, 364 (77%) of the surveys were completed and returned. The survey, which was pre-tested on 40 farmers and consisted of 18 questions, which could be both open-ended and closed-ended, inquired about herd characteristics and reproductive management. In addition to the survey, data were extracted from the SOMRS and reproductive measurements and other performance values were obtained for each farm as an average for the year 2005/2006. The association between VWP and measurements of herd reproductive efficiency was investigated with a linear regression model.

Results 53 of the herds said that they did not have a specific strategy for VWP while the average VWP were close to 60 days (Table 1). The interval of days from the end of the VWP to the herd average first AI (Table 2) was on average 28 and 23 days respectively. The interval decreases when the VWP increases. Most farmers replied that they chose a VWP according to the targeted calving interval (Table 3).

Table 1 Voluntary waiting period, in days, as answered by the farmers (IQR=Inter quartile range)

	Number of replying herds	Mean	Median	IQR
1st lactation	306	66.5	60	50-80
2nd lactation>	304	59.5	57	50-60

Table 2 Linear regression models of interval of days from end of voluntary waiting period (VWP) to herd average first AI (FAI) on the VWP.

	Intercept	Parameter
1st lactation	54.4	-0.42 x (VWP)
2nd lactation>	56.6	-0.41 x (VWP)

Table 3 Answers (number of herds) to the question on why the herds wanted a certain voluntary waiting period

Answer	1st lactation	2nd lactation
A: Gives the wanted calving interval	121	137
B: Tradition, have always done it like this	66	53
C: Its when I observe the first oestrus	50	70
A+B	10	9
A+C	3	2
B+C	4	8
Other	76	46

Conclusion The target VWP still seems to be around 60 days, but may be a little longer for 1st lactation cows, although, on average it seems that it takes about one oestrus cycle after the VWP to perform the first AI. Most herds have a strategy for the VWP and most of them answered that they choose this VWP to obtain their preferred calving interval.

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Estimation of genetic variation in embryo quality: Is there potential to genetically select cattle with the inherent ability to produce high quality embryos?

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Introduction Declining dairy cattle fertility and low pregnancy rates are now well documented world wide and causes are considered to be multi-factorial e.g. nutritional, managerial and genetic. The genetic component of female fertility has been investigated in part using both traditional and physiological parameters and fertility selection indexes based on traditional measures are now widely available offering the industry an opportunity to make informed breeding decisions with respect to female fertility. Although researchers have come a long way in understanding the genetic variation in fertility, traditional measurements, although practical to measure have low heritability and do not indicate in detail where or why the reproductive system is actually failing. On the other hand, physiological measurements are more representative of the cow's inherent ability to be fertile and consequently are less influenced by management and thus tend to have a higher level of heritability. Poor embryo development and survival is suggested to be one such cause of declining fertility (Boland, 2005). Multiple ovulation embryo transfer (MOET) is widely carried out by breeding companies and farmers to produce multiple offspring from animals of high genetic merit. The refinement of the technique and development of equipment has improved the success of embryo transfer (ET), however, the success of ET is affected by many factors including the technician, farm, recipient and embryo quality (Hasler, 2003). To our knowledge there is no published literature on the genetic variation present in embryo quality. Therefore the objective of this work was to estimate genetic variation in embryo quality and to assess a number of the factors affecting it.

Materials and Methods Records were available for 1577 embryo's collected during 2002 at one farm from 401 superovulated Holstein-Friesians heifers (12-16 months of age) taking part in a multiple ovulation and embryo transfer breeding program (MOET). The embryos formed 65 paternal half-sib families varying between 2 and 231 embryos/sire. Embryos were graded immediately after collection by skilled technicians according to the international embryo transfer society (IETS) embryo quality grading scale (1 = excellent to 3 = poor; Stringfellow & Seidel, 1998). Univariate mixed linear models were fitted to the data using ASREML software (Release 2.0 VSN International. Ltd., UK). The inclusion of various effects in the model was explored and the final model included the fixed effect of technician (n = 3) and semen type (frozen; normal and sexed semen). Embryo sire (n = 65) was fitted as a random effect. The heritability was calculated as the additive genetic variance (sire variance * 4) divided by the phenotypic variance (genetic + residual variance).

Results The genetic variation present in embryo quality is significant and of additive genetic nature ($h^2 = 0.13$; Table 1). Furthermore, both technician and semen type (normal or sexed) had a significant effect ($P<0.001$) on the embryo quality. The significance of the effect of semen type implies that on average, the quality of embryos produced from animals within the MOET breeding program using normal semen is higher (IETS grade average 1.15 se 0.07) than when using sexed semen (IETS grade average 1.50 se 0.02). The effect of technician is not surprising and is most likely to be due to the subjective nature of the embryo grading process.

Table 1 Number of records, mean and heritability (h^2) of embryo quality grade

Parameter	n	Mean (s.e.)	h^2 (s.e.)
Embryo Quality	1455	1.22 (0.01)	0.13 (0.06) **

$P<0.05=^{*}$; $P<0.01=^{**}$; $P<0.001=^{***}$

Conclusion Whilst the dataset here is small, the analyses has indicated that significant genetic variation is present in embryo quality and suggests a moderate heritability similar in magnitude to that of a number of type and production disease traits. Therefore it appears that some sires are able to produce embryos of a significant higher quality than others offering the potential to improve embryo quality through genetic selection. The implications of these results could be vast in terms of the impact this may have on subsequent fertility and may also be of interest to other species including humans. Furthermore, the effects of semen type (normal or sexed) on embryo quality imply that the use of sexed semen in cows having undergone superovulation such as those within a MOET breeding scheme may be likely to yield embryos of reduced quality. This may help explain the difference in pregnancy rates between conventional and sexed-semen observed throughout the world. Further detailed analyses of a larger dataset is needed to confirm these findings and to further investigate other factors involved in the production of embryos.

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Acknowledgements Cogent Breeding Ltd for provision of the data.

Comparison of ovarian follicles at follicle wave emergence between heifers with high versus low numbers of follicles

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Introduction Recent studies in cattle demonstrate that the numbers of antral follicles present on ovaries during follicular waves vary greatly among animals, but are highly repeatable within individuals. Although relatively high numbers of antral follicles are positively associated with fertility in numerous species, the causes and physiological significance of the high variation in follicle number during follicular waves in cattle is not known. Antral follicle numbers are inversely associated with serum FSH concentrations in animals of high versus low follicle number phenotype, but no differences in serum oestradiol are present. However, differences in FSH concentrations in animals of the two follicle number phenotypes may still be reflected by differences in estrogenic capacity of ovarian follicles collected early in a follicular wave. Thus, the purpose of this study was to examine indicators of estrogenic capacity of ovarian follicles collected from such animals at wave emergence via comparison of follicular fluid steroid (oestradiol and progesterone) concentrations, and mRNA abundance for the aromatase enzyme and LH and FSH receptors in follicles from heifers with low versus high numbers of follicles.

Materials and methods Cross-bred beef heifers (n=32) were synchronized with two injections of prostaglandin (PG) given 11 days apart. Ovarian follicle development was monitored on a daily basis in each heifer by transrectal ultrasonography. Heifers were then assigned to one of three groups based on the peak number of follicles (≥3 mm in diameter) per wave: Low (≤14 follicles, n=5), Intermediate (15≥26 follicles, n=22) or High (≥27 follicles, n=5). Animals in the Low and High group were slaughtered at emergence of the first follicle wave (12 to 24 hrs after ovulation). Pairs of ovaries were collected and weighed. All follicles ≥3 mm were dissected from both ovaries and diameter was measured. Follicular fluid and granulosa and theca cells were isolated. Follicles were classified individually within animal (F1 to F6) based on decreasing follicle diameter. When two follicles had the same diameter they were classified based on follicular fluid oestradiol concentrations). For data analysis, follicles were also stratified into one of three size classes (4-4.5 mm, 5-5.5 mm and ≥6 mm). Oestradiol and progesterone concentrations in follicular fluid were measured by RIA and levels of mRNA for aromatase and FSH and LH receptors were measured by quantitative RT-PCR (and were expressed relative to β-Actin).

Results Total weight of ovaries per animal was greater ($P<0.05$) in heifers with high versus low numbers of follicles (15.5±2.09 v 9.5±0.70 g). Diameters of follicles classified into a given size category (F1 to F6) were not different between groups. The follicular fluid oestradiol concentrations of the largest (F1) and the second largest (F2) follicles were greater ($P<0.05$) in heifers with low versus high numbers of follicles (115±20.9 v 48.6±11.3; 106.1±18.6 v 40.5±10.3 ng/ml, respectively). Also heifers with low numbers of follicles had greater ($P<0.05$) oestradiol: progesterone ratio in the F1 follicles than similar follicles in heifers with high numbers of follicles (4.8±1.0 v 2.3±0.9). When data were analysed relative to follicle size classes, follicular fluid oestradiol concentrations were greater ($P<0.05$) in ≥ 6 mm follicles in heifers with low versus high numbers of follicles (105.3±15.9 v 41.2±10.2 ng/ml, n=10 v 7). The E:P ratio was greater ($P<0.05$) in follicles in the 5-5.5 mm and ≥6 mm size classes in animals from the Low group compared to the High group (6.6±2.6 v 1.8±0.53, n=8 v 12; 4.0±0.7 v 1.9±0.6, n=10 v 7).

In granulosa cells, levels of mRNA for aromatase were greater ($P<0.05$) in F1 (0.26±0.09 v 0.08±0.04), F2 (0.15±0.04 v 0.06±0.01) and F3 (0.07±0.03 v 0.02±0.008) follicles in animals with low versus high numbers of follicles. mRNA levels for LH and FSH receptors were not different ($P>0.05$) between animals in the Low and High groups. In theca cells, mRNA levels for LH receptors were higher in the F2 (0.009±0.002 v 0.003±0.002) and F3 (0.005±0.001 v 0.014±0.004) follicles from heifers with low compared to high numbers of follicles, but there was no difference between F1 follicles (0.022±0.004 v 0.026±0.012).

Conclusion Heifers with low numbers of ovarian follicles have smaller ovaries, and their follicles about the time of follicle wave emergence have higher follicular fluid oestradiol concentrations and more mRNA for the aromatase enzyme. The causes and significance of these differences remains to be established. Whether such differences in the intrafollicular steroid environment of animals of the two follicular phenotypes ultimately influence other subsequent ovarian/physiological parameters linked to fertility remains to be investigated. However, we suggest that follicles from animals of low follicle number phenotype are more oestrogen active and therefore may be of much better quality, and contain better quality of oocytes and produced better quality embryos than the follicles from animals of high follicle number.

Controlling age and size of the ovulatory follicle in lactating dairy cows

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Introduction Fertility of dairy cows is at a critical all time low and its decreasing trend continues at an alarming pace. Recent studies from our laboratory and others indicate strong observational associations between size, age and function of the ovulatory follicle and the probability of successfully establishing a pregnancy in lactating dairy cows (Bello *et al*, 2006, Bleach *et al*, 2004). We are currently developing a model to test a cause-effect relationship between age and size of the ovulatory follicle, and fertility. The purpose of this study was to determine if age and size of the ovulatory follicle could be controlled following follicular emergence by modifying time to induction of luteolysis.

Materials and methods This study was conducted between August 2006 and March 2007 at the Michigan State University Dairy Teaching and Research facility. Lactating dairy cows (n=49) between 50 to 100 days in milk were treated with G6G Pre-Ovsynch (Bello *et al*, 2006) + Ovsynch (Pursley *et al*, 1995) without the final injection of gonadotropin releasing hormone (GnRH). All cows were treated as follows: 25 mg prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, Pre-P; Prostamate, IVX Animal Health Inc.), 2 d later 100 μg GnRH (Pre-G; Ovacyst, IVX Animal Health Inc.), then 6 d later 100 μg GnRH (1st GnRH of Ovsynch). Then, cows were blocked by parity and randomly assigned to receive two 25 mg injections of $PGF_{2\alpha}$ (induction of luteolysis) 12 h apart at either 5 (CL d5), 6 (CL d6) or 7 (CL d7) d following 1st GnRH of Ovsynch (Figure 1).

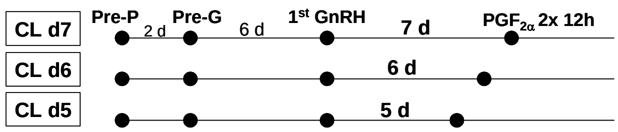

Figure 1: Representation of treatment scheme.

Ovaries were monitored daily with ultrasonography from 1st GnRH of Ovsynch until 60 h after injection of $PGF_{2\alpha}$ of Ovsynch. Then ovaries were monitored at 4 h intervals until ovulation for a maximum of 140 h after $PGF_{2\alpha}$. Criteria for inclusion in the study included response to G6G Pre-Ovsynch (luteolysis followed by ovulation) and ovulatory response to 1st GnRH of Ovsynch, followed by emergence of a new follicular wave. Size of the ovulatory follicle at the time of $PGF_{2\alpha}$ of Ovsynch and maximum size of the ovulatory follicle within 24 h of detected ovulation were compared between treatments using generalized linear mixed models (MIXED procedure, SAS Version 9.1, SAS Institute Inc., Cary, NC). Age of the ovulatory follicle was defined as the interval between induction of follicular emergence (1st GnRH of Ovsynch) and ovulation. Interval from induction of luteolysis to ovulation and age of the ovulatory follicle were compared between treatments using survival analysis (LIFETEST and PHREG procedures, SAS Version 9.1).

Results Size of the dominant follicle at induction of luteolysis increased linearly across treatments of increasing luteal lifespan ($P<0.001$; Table 1). Ovulation by 140 h after induction of luteolysis was detected in 38 of the 49 cows assigned to this study. Among cows with detected ovulation, neither maximal size of the ovulatory follicle nor interval from induction of luteolysis to ovulation differed between treatments ($P=0.24$ and $P=0.46$, respectively; Table 1). Thus, age of the ovulatory follicle was decreased across treatments in a manner proportional to the reduction in the interval from 1st GnRH of Ovsynch to induction of luteolysis ($P<0.001$; Table 1).

Table 1 Size of the ovulatory follicle at induction of luteolysis and within 24 h of ovulation, interval from induction of luteolysis to ovulation, and age of the ovulatory follicle in cows induced to undergo luteal regression 5 (CL d5), 6 (CL d6) or 7 (CL d7) d after induction of follicular emergence (1st GnRH of Ovsynch).

	Treatments			
	CL d5	CL d6	CL d7	P-value
Cows, n	17	16	16	
Size of the ovulatory follicle at induction of luteolysis [1], mm	11.0 ± 0.3	11.4 ± 0.4	12.9 ± 0.3	Linear (P<0.001)
Cows that ovulated, n	11	15	12	
Maximal size of the preovulatory follicle [1], mm	16.8 ± 0.7	16.7 ± 0.4	17.6 ± 0.5	0.24
Interval from induction of luteolysis to ovulation [2], h	110.8 ± 2.9	110.8 ± 4.3	105.3 ± 1.8	0.46
Age of the ovulatory follicle [2], h	230.8 ± 2.8[a]	254.4 ± 4.3[b]	273.3 ± 1.8[c]	[a,b,c] <0.001

[1]Mean ± SEM. [2]Kaplan-Meier mean estimate ± SEM

Conclusions Treatments were designed so that induction of luteolysis occurred 5, 6 or 7 d after induction of follicular emergence (1st GnRH of Ovsynch). Thus, the observed linear effect of treatment on follicular size at induction of luteolysis was expected. Following induction of luteolysis, follicles grew to a similar preovulatory size for a similar length of time prior to ovulation. Thus, by design, treatments yielded ovulatory follicle of ages directly proportional to the length of the interval between induction of follicular emergence (1st GnRH of Ovsynch) and induction of luteolysis. In conclusion, this model permitted control over age and size of the ovulatory follicle in lactating dairy cows in peak milk production.

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Lameness, progesterone, oestradiol and LH profiles and ovarian follicular dynamics in dairy cows

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Lameness is a major disease affecting fertility in UK dairy cattle and the objective of this study was to compare hormone profiles and ovarian follicular changes in lame and non-lame cows on 2 commercial farms. Cows (n = 55) 30-70 days post partum were scored on a weekly basis for lameness (scale 1-5; increasing in severity). Milk sampling and trans-rectal ultrasonography were undertaken daily during and after oestrus synchronization (GnRH followed 7 days later by PGF2α). Maximum diameter of the dominant follicle was the same in lame and non-lame cows ($p = 0.67$; $n = 55$), however, fewer lame cows ovulated (26/37 lame *versus* 17/18 non-lame; $p = 0.042$). A subset of 17 animals was also frequently blood sampled for LH pulse and surge analysis. Of these, all 12 (100% cows that ovulated (4 non-lame and 8 lame) had a marked LH surge while the remaining 5 lame cows did not. In the lame animals, LH pulse frequency also differed between ovulated and non-ovulated animals (0.76 pulses/h versus 0.53 pulses/h; $p = 0.012$, $n = 13$). LH pulse frequency was positively correlated with plasma oestradiol concentration both at the time of pulse sampling (approx. 37-28h before LH surge; $p = 0.013$) and in the full period 30h before the LH surge ($p = 0.027$). We conclude that lame cows with low LH pulse frequency are *more* likely to have lower oestradiol concentrations in the late follicular phase and are *less* likely to have an LH surge and ovulate.

Supported by the Wellcome Trust and Universities UK.

A survey on fertility in the Holstein populations of the world

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Introduction In 2006 the World Holstein Friesian Federation (WHFF) initiated a survey of the status on fertility in the Holstein populations around the world. The background was numerous reports of Holstein cows and heifers having problems getting pregnant. The board of WHFF assigned a working group to generate the questions for the survey.

Methods The questions in the survey included questions on descriptions of fertility measures recorded, phenotypic and genetic trends, comparison with other dairy breeds, weight on fertility in the overall selection index, Interbull initiative, sexed semen, synchronized breeding, genomics of fertility, and more subjective questions on the "fertility problem".
The survey was then sent to a number of Holstein federations around the world. By the end of April 2007, 19 national Holstein associations had responded. Not all countries had responded to all questions, but to most.

Results and discussion Most countries believe that the dairy industry currently has the necessary tools to help farmers improve the genetic potential of their cows' fertility. However, a few countries pointed to the lack of recording of fertility traits or indicator traits. Most countries also believed that the four traits Interbull is working on will give a good basis for selection on overall fertility performance.
In the 19 countries 8 different fertility measures were used for heifers (nulliparous) and 18 fertility and indicator measures were used for cows. Only 9 countries used heifer information in the genetic evaluation. Of the measures on cows, the interval from calving to first insemination (13 countries), calving interval (13 countries), number of inseminations to achieve conception (11 countries), non-return rate 56 days after insemination (9 countries), and number of days open (8 countries) were the most used. The excessive number of traits makes comparison across countries difficult. It has also caused an enormous need of work from Interbull.
The 5 most used fertility measures decreased on average in the countries reporting trends, both phenotypically and genetically. Twelve of 19 countries reported the genetic trend for their national overall measure of fertility. The answers ranged from markedly getting worse to markedly improving. On average a slightly unfavorable trend was reported. This confirms the concerns of Holstein breeders, and highlights the need for action.
Eight countries reported the correlation between their national overall measure of fertility and their total merit index. The values were between 0.03 and .40 and the average was 0.15. So despite positive correlations between fertility and total merit, fertility tends to decrease. This can occur because bull dams have very low accuracies for fertility, so production tends to be the reason for their selection. Hence, selection pressure on fertility only comes from selection of sires of sons.

Conclusion The results of this survey suggest that fertility so far has been a problem in the Holstein populations around the world. Action needs to be taken, both internationally as well as within each country, in order to change the decline of fertility. Many countries already have genetic evaluation for fertility traits. But management also needs to be improved in the increasingly larger herds.

Risk factors for perinatal calf mortality in the Irish national Holstein-Friesian dairy cow population

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Introduction Perinatal mortality is a significant cause of reproductive loss. Recent studies in Denmark, The Netherlands, North America and Sweden indicate that the prevalence of bovine perinatal mortality is increasing, particularly in Holstein primiparae. Traditionally, over 50% of this mortality has been directly attributed to dystocia (Mee, 1999). However, there is now some evidence to suggest that an increasing proportion of perinatal mortality occurs at eutocia (idiopathic stillbirth or weak calf syndrome) where placental dysfunction and low birth weight may be causative factors (Kornmatitsuk *et al*., 2004). This raises the question as to whether the risk factors conventionally associated with perinatal mortality may be of greater or lesser importance now and differ in pasture-based systems. This study was carried out to establish the national prevalence of perinatal mortality in heifers and cows on commercial dairy farms in Ireland and to determine the current significance of putative risk factors in pasture-based management systems.

Materials and methods Data on calf, sire and dam identification number, calving date, calf gender, degree of calving assistance, occurrence of perinatal mortality (recorded as calf dead at birth or within 24-hours), age, parity and breed of the dam and breed of service sire were extracted from the Irish Cattle Breeding Federation database for the years 2002 to 2005. Only births (single or twin) from dams who were at least 50% Holstein or Friesian and which were mated to AI sires whose main breed component (i.e., >50% their genes) was Angus, Belgian Blue, Charolais, Hereford, Holstein/Friesian, Limousin or Simmental Holstein-Friesian were retained. Predicted transmitting ability (PTA) of the service sire for perinatal mortality was obtained from the August 2006 genetic evaluations. Herd-years with less than 20 calving records were removed and the prevalence of perinatal mortality was calculated from a total of 182,026 records. Perinatal mortality in the previous calving, within dam, was also retained. The logit of the probability of a perinatal mortality was modelled using multiple regression generalised estimating equations in PROC GENMOD (SAS, 2006). Interactions between variables were also tested for significance in the model where significance was declared at $P<0.001$ based on the GEE score statistic.

Results The prevalence of perinatal mortality in this dataset was 4.29% (7.7% in primiparae and 3.5% in pluriparae). Twin calving occurred in 2.72% of calvings. The percentage of calvings scored as no assistance/unobserved, slight assistance, severe assistance and veterinary assistance (including Caesarean) was 57.4, 19.2, 3.7 and 2.2%, respectively; 17.5% of records had no data on calving assistance. The likelihood of perinatal mortality was significantly higher in male calves, assisted and difficult calvings, twin calvings, primiparous calvings, dams (particularly primiparae) calving at a young age relative to the median within parity, whether the dam had a perinatal mortality in the previous calving or not, and from service sires with a positive PTA for perinatal mortality. Interactions were evident between parity and a quadratic regression on age at calving, relative to the median within parity, between parity and degree of calving assistance and between parity and multiple births. Breed of sire or herd size were not significant risk factors. The likelihood of perinatal mortality increased, across all animals, over time, albeit in a short period (four years).

Conclusion These results indicate that although the prevalence of perinatal mortality in the Irish dairy cow population has recently increased, the current prevalence is similar to that in other pasture-based dairy systems worldwide and lower than that in confinement systems. The putative risk factors traditionally associated with perinatal mortality were still associated with perinatal mortality in these commercial dairy herds. In order to reduce the risk of perinatal mortality in Holstein-Friesians, herdowners must focus on the key risk factors largely under their control; age at first calving, degree of calving assistance and sire PTA for perinatal mortality.

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Field investigation of perinatal mortality in Friesian cattle associated with myocardial degeneration and necrosis

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Perinatal mortality and stillbirths were investigated in an extensively managed herd of Friesian cows and heifers calving over a two-year period: 504 cows were calved and 215 heifers. Cows were bred to Friesian and Jersey bulls using natural service. The perinatal fetal mortality rate for cows was 7.5% and 30% for heifers. An experienced stockman managed these calvings and veterinary assistance was required on only 9 occasions. All stillborn fetuses were examined *post mortem* by the Veterinary Laboratories Agency, Carmarthen and no infectious agent was identified on any occasion. Thyroid hyperplasia was found in two fetuses. In blood samples taken from 10 late pregnant heifers, mean glutathione peroxidase values were 7.9 ± 1.7 iu/ml rbcs. Tissues from eight fetuses were submitted to the University of Liverpool for histopathological examination; all presented lesions consistent with myocardial degeneration and necrosis of the left ventricle. Following treatment of 205 late pregnant heifers with sodium selenite and vitamin E the overall perinatal mortality rate in these cattle fell to below11%. The role of selenium/vitamin E related to cattle fertility will be discussed.

Effects of CLA supplementation on milk production and reproductive performance of dairy cows

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Introduction For dairy cows, the early lactation period is associated with negative energy balance (NEB) and consequent body reserve mobilisation, as the energy required for maintenance and milk energy output exceeds dietary energy intake. Numerous reports have indicated that the duration and extent of NEB have negative effects on onset of cyclicity and pregnancy rates (Beam and Butler, 1999, Diskin, et al., 2003). Trans-10, cis -12 conjugated linoleic acid (CLA) is a geometric and positional isomer of linoleic acid that reduces mammary milk fat synthesis in a dose-dependent manner (de Veth et al., 2004). Potential beneficial effects of CLA supplementation on fertility indices have been reported (Castaneda-Gutierrez, et al., 2005). This study was carried out to examine the effects of CLA supplementation during early lactation on milk production and reproductive indices.

Materials and methods Seventy-two Holstein-Friesian cows (32 primiparous and 40 multiparous) that calved between August and November 2006 were used in a completely randomized block design. Cows were blocked on the basis of expected calving date, previous lactation yield and body condition score (BCS), and randomly assigned to receive either 60 g per day of lipid encapsulated CLA (LE-CLA; BASF AG, Ludwigshafen, Germany) or 60 g per day of calcium salts of palm fatty acids (CSFA) from parturition until 60 days in milk. The LE-CLA contained a 50:50 mix of cis-9, trans-11 CLA and trans-10, cis-12 CLA, resulting in a daily intake of 6 g per day of each isomer. Both the LE-CLA and CSFA supplements were incorporated into a concentrate pellet and fed through automatic feeders (2 kg/cow day^{-1}). The basal diet was fed ad libitum and consisted of 0.5 grass silage, 0.25 soya hulls, 0.15 ground barley, 0.09 soyabean meal, and 0.01 vitamins and minerals. Milk yield and dry matter intake were measured daily. Milk composition, bodyweight and BCS were recorded weekly. The breeding season lasted from 28/11/06 to 27/02/07. Following a 40 day voluntary waiting period after calving, cows were inseminated following visual observation of oestrus. Daily milk production and dry matter intake data were collapsed into weekly means for analysis. Data analysis was carried out using the MIXED procedure of SAS with repeated measures and an autoregressive covariance structure. Treatment, lactation week, and the interaction between treatment and lactation week were used as fixed effects, and block was included as a random effect. Lactation number and calving day of year were included in the model as adjustment variables. Binary variables were analysed using the chi-square test.

Results There was no difference between LE-CLA and CSFA treatments in milk yield during the first 8 weeks of lactation (24.4 vs. 24.6 kg/day; $P = 0.8$), but milk fat concentration was reduced in the LE-CLA group (41.1 vs 44.2 g/kg; $P < 0.05$), with maximal milk fat depression of 14% occurring at week 8 (35.6 vs. 41.5 g/kg; $P < 0.01$). Additionally, during lactation weeks 5 to 8, daily milk energy output was lower ($P < 0.05$) for LE-CLA cows compared to CSFA cows (19.9 vs. 20.9 MCal/day). Body condition score declined in both treatments for the first 4 weeks of lactation. Thereafter LE-CLA cows did not loose any further BCS whereas CSFA cows continued to mobilise body reserves (week 5 to 8 BCS: 2.96 vs. 2.83; treatment by time, $P < 0.05$). Conception rate to first service tended to be higher for cows on the LE-CLA treatment (51.5 vs. 38.9 %; $P = 0.11$), and the number of services per conception also tended to be reduced (1.72 vs. 2.00; $P = 0.07$).

Conclusions Previous studies have indicated trends for improved reproductive performance for cows supplemented with LE-CLA, and our observations are broadly in agreement with those findings. In contrast to earlier reports that have shown an increase in milk yield following LE-CLA supplementation, no increase in milk yield was observed in the current study. Instead, it appears that the energy spared in reducing milk energy output was retained, resulting in improved BCS. This alteration in nutrient partitioning may explain, at least partially, the trend for improved reproductive performance. Further work is warranted with greater cow numbers to evaluate the effects of LE-CLA on dairy cow fertility.

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Conjugated linoleic acids and the dairy cow

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Introduction. In the last decade there have been significant advances in our understanding of rumen lipid metabolism and the potential biological effects of rumen-derived 'bioactive' fatty acids. We now recognize that certain fatty acids produced in the rumen can have specific and potent effects on ruminant metabolism and human health. Of particular interest are conjugated linoleic acid (CLA) isomers, which are produced during rumen biohydrogenation of dietary polyunsaturated fatty acids. The objectives of this workshop are to provide an overview of the biology of CLA in the dairy cow, highlight the biological effects of CLA isomers and discuss the potential use of rumen-protected supplements of CLA in early lactation. Of particular focus is the exciting potential of supplemental dietary CLA as an approach to improve reproductive performance.

Conjugated linoleic acid isomers. The term CLA refers to a mixture of positional and geometric isomers of linoleic acid with a conjugated double bond system; milk fat can contain over 20 different isomers of CLA. Many biological activities have been reported for CLA isomers; *cis*-9, *trans*-11 CLA (rumenic acid; RA) and *trans*-10, *cis*-12 CLA have been the most investigated, with their presence in ruminant fat (including milk fat) a consequence of rumen biohydrogenation. RA represents 75 - 90% of total CLA in ruminant fat and is of interest as a potential functional food component because biomedical studies with animal models indicate RA has anticarcinogenic and antiatherogenic properties. Under certain dietary conditions *trans*-10, *cis*-12 CLA is also a rumen biohydrogenation intermediate.

Under particular dietary situations, a reduction in the content and yield of milk fat occurs in dairy cows. This has commonly been referred to as milk fat depression (MFD) and recent investigations indicate that this is related, at least in part, to effects of specific CLA isomers on rates of milk fat synthesis. *Trans*-10, *cis*-12 CLA is a potent inhibitor of milk fat synthesis and its mechanism involves a coordinated reduction in mammary expression of genes for key enzymes in the pathways of milk fat synthesis. Effects of *trans*-10, *cis*-12 CLA are specific for milk fat, and there is a curvilinear relationship between the dose of *trans*-10, *cis*-12 CLA and the reduction in milk fat synthesis; as little as 2.5 g/d leaving the rumen is sufficient to cause a 25% reduction in milk fat production. To date, *trans*-10, *cis*-12 CLA is the only biohydrogenation intermediate that has been unequivocally identified to inhibit milk fat synthesis, but recent studies have identified two additional CLA isomers that appear to regulate milk fat synthesis, *trans*-9, *cis*-11 and *cis*-10, *trans*-12 CLA. The rumen formation of these three CLA isomers and their subsequent uptake by the mammary gland may be sufficient to account for most situations of dietary-induced MFD.

One of the exciting aspects of the CLA research has been the broader implications; in addition to applying this knowledge in dairy production to maintain a normal milk fat production when that is desirable, dietary supplements of *trans*-10, *cis*-12 CLA can also be used as a management tool for reducing milk fat in a controlled manner. Milk fat is the major 'cost' of milk synthesis accounting for over one-half of the energy needed for milk synthesis by the animal; consequently, a controlled reduction in milk fat output will result in a sparing of nutrients that can be used for other purposes. The ability to selectively reduce milk fat yield could be of economic value in situations where there is a quota on milk fat yield. It could also benefit cow well-being by reducing energy demands during times when nutrient intake is inadequate, such as during environmental stress or in the transition period at parturition.

Rumen protected supplements of CLA. Commercial application of CLA as a management tool requires a CLA formulation that must have two characteristics; it must offer protection of the CLA from alterations by rumen bacteria and the CLA must subsequently become available for absorption in the small intestine. Originally, the majority of research on rumen-protected CLA used supplements consisting of calcium salts of free fatty acids. Recently, the use of a lipid-encapsulation technology has been developed as a process to protect CLA from rumen metabolism. This process coats the bioactive ingredient in question (i.e. CLA isomers) with hydrogenated soybean oil, which consists predominately of stearic acid (18:0). The encapsulation of the CLA isomers within saturated fatty acids provides a physical protection from rumen biohydrogenation while still allowing these fatty acids to be absorbed post-rumen. A lipid-encapsulated CLA supplement is now commercially available in Europe (Lutrell®, BASF AG, Ludwigshafen, Germany). This supplement contains a 50:50 mixture of RA and *trans*-10, *cis*-12 CLA. A consistent reduction in the level of milk fat has been observed in studies using 'rumen-protected' supplements of CLA over treatment periods ranging from 3 to 20 weeks involving primiparious and multiparious cows at different stages of lactation and under different dietary and management practices. Under some dietary situations the sparing of energy by reducing milk fat synthesis has allowed the repartitioning of nutrients to support increased milk and milk protein output. Importantly, in many situations in which CLA supplements have been fed to the transition dairy cow, an improvement in reproductive performance has been observed. Based on these observations, recent research focus has concentrated on the potential avenues through which supplemental RA and *trans*-10, *cis*-12 CLA may impact the metabolism and function of the ovary and uterus whether this be through sparing nutrients which may be used for other purposes and/or nutritive signaling. The available data in this area will be expanded upon in the subsequent presentation.

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Conjugated linoleic acids and reproductive performance of dairy cows.

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Introduction. Dairy cow reproductive performance has declined in recent decades, but the precise mechanisms responsible have not been fully elucidated. Nevertheless, conjugated linoleic acids (CLA) could improve reproductive performance through a number of possible mechanisms. The reports referenced in this summary used supplements containing equal amounts of the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA isomers, and hereafter will be referred to as CLA.

Energy Balance. It is well documented that early postpartum negative energy balance (NEB) and body condition score loss are associated with compromised reproductive performance during the subsequent breeding period. Fat is the most energetically expensive component of milk, and hence represents a major energetic burden during the early lactation period. *Trans*-10, *cis*-12 CLA is a rumen biohydrogenation intermediate that when taken up by the mammary gland inhibits milk fat synthesis in a dose dependent manner. Feeding this compound during early lactation NEB could have an energy sparing effect by reducing milk fat output. However, many reports with high-producing dairy cows indicate that energy spared by reducing milk fat concentration is partitioned to increasing milk volume (Bernal-Santos et al., 2003; Odens et al., 2007), but this is not consistently observed (Castaneda-Gutierrez et al., 2005; Moore et al., 2004). Most studies with high yielding cows report no change in EB status with CLA supplementation during the transition period (Bernal-Santos et al., 2003; Castaneda-Gutierrez et al., 2005; Moore et al., 2004), though higher supplementation rates have been shown to improve EB (Odens et al., 2007). In a recent study with cows of moderate milk production potential, a significant improvement in postpartum energy balance and reduced postpartum body condition score loss was observed in TMR-fed cows supplemented with CLA (Butler et al., unpublished). Similarly, the energy balance status of early lactation grazing dairy cows was improved by CLA supplementation (Kay et al., 2006).

Circulating progesterone concentrations. Progesterone is essential for establishment and maintenance of pregnancy. A number of studies have demonstrated that cows that conceive following insemination have a more rapid postovulatory rise in progesterone (Stronge et al., 2005). Though just a single report exists, a recent study indicated that cows supplemented with CLA had increased circulating concentrations of progesterone (Castaneda-Gutierrez et al, 2007). Their report also indicated that CLA supplementation increased circulating IGF-I, and this may have been responsible for the increased progesterone concentration. It is also possible that the clearance rate of progesterone was reduced by CLA supplementation, as has been previously reported for cows fed supplemental fat (Hawkins et al., 1995).

Endometrial prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) synthesis. Maternal recognition of pregnancy in the cow occurs around days 15-17 after insemination. In the absence of a sufficient interferon-τ signal from the developing embryo, luteal pulses of oxytocin will stimulate endometrial $PGF_{2\alpha}$ release, thus inducing luteolysis and a return to oestrus. Much of the decline in reproductive performance in the modern dairy cow is due to early embryo mortality (Diskin et al., 2006), and some of this loss is likely due to inadequate or borderline embryonic interferon-τ synthesis. It is possible that reducing endometrial $PGF_{2\alpha}$ synthesis and release could increase the likelihood of conception. In vitro incubation of bovine endometrial cells with CLA isomers reduced $PGF_{2\alpha}$ production (Mann et al., 2006; Rodriguez-Sallaberry et al., 2006). However, in vivo oxytocin challenges have not shown any difference in PGFM between CLA supplemented and control cows (Castaneda-Gutierrez et al., 2007).

Conclusions. Though most studies to date have had inadequate animal numbers to properly test effects on reproductive performance, improved indices of reproductive performance were observed with high (Bernal-Santos et al., 2003; Castaneda-Gutierrez et al., 2005; Mann et al., 2007) and moderate yielding cows (Butler et al, unpublished). It is possible that the improved reproductive performance may be mediated at least partially by improved energy status, but it is also likely that CLA isomers have direct effects independent of effects on EB. These could include effects on circulating progesterone (luteal secretion and/or progesterone clearance), reduced endometrial secretion of $PGF_{2\alpha}$, and a number of areas that have yet to be researched, including oocyte quality, uterine environment, rate of embryo development, and conceptus-maternal interaction. Further work is necessary to fully elucidate the potential mechanisms responsible for the fertility-improving effects of CLA in dairy cows.

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Where did she come from? Where is she going? – The potential of on-farm fertility profiles

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Introduction

Traditional monitoring of reproductive status, i.e. visual observation, is equivalent to observing selected single frames from a film. Serial measurements and Time-series analysis allow the whole film to be followed. This paper explores the possibilities for improved reproductive management made possible by the availability of time-series measurements. We focus on progesterone time-series as these are now becoming available for the first time in-line as an automated system, and activity meters as a representative of the behavioural manifestations of oestrus.

Real-time measurements empower real-time decision-making

An important issue for reproductive management is whether or not to inseminate to a given oestrus. Real-time measurements allow this issue to be evaluated as the cow is coming into oestrus. From the biological viewpoint the issue is: what is the likelihood of a prospective insemination being successful? We have previously developed algorithms for calculating a likelihood of a prospective AI being successful (LikeAISucc) based on characteristics of the progesterone time-series supplemented with additional information, e.g. prior disease (Friggens and Chagunda, 2005). The LikeAISucc function has now been tested in a progesterone dataset containing 600 lactations. The LikeAISucc function is one example of the way in which a real-time time-series can empower real-time decision-making.

Combining reproductive measures

We could expect an improvement in the algorithm predicting LikeAISucc if information on the behavioural strength of the oestrus is included. This could be done by combining information from activity meters with the progesterone time-series. Having both in-line progesterone and activity meters may seem like a luxury situation, after all who would invest in two systems to detect the same thing? Actually, combining them provides extra information, and maybe can save money on recurrent costs. Obviously, having progesterone means that the false positives associated with activity measures can be readily identified. Likewise, once the cow has been progesterone identified as coming into oestrus, activity meters can improve the timing of inseminations relative to the information from progesterone. Implicit in this is the assumption that progesterone is a truer indicator of reproductive status. This opens up the possibility of using activity meters as one factor driving the progesterone sampling. This allows a low basal progesterone sampling frequency that can be ramped up when an activity spike occurs. This is valuable during periods when the progesterone level is essentially stable, especially during pregnancy. After the first 25-30 days post-insemination, checking whether a cow is still pregnant by using progesterone alone requires that samples be taken during a pre-chosen 20-25 day window. However, if activity meters are in use then the monitoring for pregnancy failure, i.e. return to oestrus, can be done using the activity meters to trigger a progesterone sample if a pregnant cow shows high activity. A similar logic can be applied to monitoring the postpartum anoestrus period.

Possibilities for future research, herd management, and breeding

Time-series measurements, if sufficiently detailed and/or combined across measurement types, remove the uncertainties associated with "snapshot" measures of reproductive status. Herd statistics, breeding values and reproductive efficiency measures are all seriously affected by the effectiveness of oestrus detection. This has been shown to vary substantially from farm to farm. This is probably the main reason for the low heritability for fertility evaluated from herd data (h^2 ~0.03). Alternative measures for fertility such as CLA determined from progesterone, or from activity measures, have substantially higher heritability (h^2 ~0.17 (e.g. Løvendahl and Chagunda, 2006)). Clearly, breeding programs would benefit from including this source of unbiased information in the assessment of female fertility. The elimination of the uncertainty in oestrus detection by using time-series measurements, together with the additional information provided by these systems, has great potential for increasing the precision of estimates of reproductive efficiency and enhancing our ability to tackle specific research questions. Realizing this potential depends upon being able to meet the challenges of analysing and interpreting multivariate time-series. This will require the development of statistical tools and as these time-series technologies become widespread requires the means to store and manage such data in central databases.

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Can dietary fatty acid supplementation aid reproduction? Challenges and opportunities.

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Introduction. Feeding fats to aid dairy cow reproduction is of considerable interest at the present time, both to scientists and the agricultural industry. This interest is based on several reasons; first, the well documented reduction in reproductive performance of dairy cows throughout the world has driven the development of nutritional strategies to reverse this trend; second, the use of dietary fat supplements will intensify as nutritionists strive to increase the energy density of diets to meet requirements of the high producing dairy cow; and third we now recognise that fatty acids (FA), both of dietary and rumen origin, can have specific and potent effects on ruminant metabolism. The objective of this paper is to provide an overview of lipid metabolism in the dairy cow and how it relates to dairy cow reproduction. Our focus will include biological processes and quantitative changes during the metabolism of FA in the rumen and the effect this has on FA availability to the dairy cow and the various areas in which FA can impact reproductive processes.

Dietary Fats and Rumen Lipid Metabolism. As well as being derived from specific fat supplements, FA in the dairy cow's diet are also present in forages and concentrates. Each fat source is composed of a different mix of individual fatty acids. Generally, most cereal grains and seeds contain a high concentration of linoleic acid (18:2 n-6), whereas linolenic acid (18:3 n-3), is typically the predominant fatty acid in forage sources. For example, corn, cottonseed, safflower, sunflower, and soybean oils are high in linoleic acid, whereas linseed is high in linolenic acid. Fish oil contains eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), two very long chain n-3 FA. Extensive metabolism of dietary lipids occurs in the rumen and this has a major impact on the profile of fatty acids available for absorption and tissue utilization. This is a result of bacterial biohydrogenation (BH) of unsaturated FA, resulting in the conversion of unsaturated FA to saturated FA, mainly stearic acid (18:0), through a series of BH intermediates (conjugated 18:2 and *trans* 18:1 FA). The major substrates are linoleic and linolenic acids and the rate of rumen BH is in the range of 70-95% and 85-100%, respectively; thus stearic acid, under typical feeding situations is the predominant fatty acid available for absorption by the dairy cow. This extensive metabolism by the rumen bacteria has made the study of dietary fat effects on reproduction challenging.

Effect of Fat Supplementation on Reproduction. Available scientific literature shows that a variety of fat supplements have benefited conception rates of lactating dairy cows. Feeding fat is a common strategy to increase the energy density of the diet; the energy status of the cow, however, is usually not improved because of a slight to moderate depression in feed intake and/or an increase in milk production. As a result, in many situations an improvement in reproductive performance has occurred without improving the energy status of the experimental animals. Therefore, fat supplementation is likely improving reproductive performance via effects of specific FA impacting metabolism and function of the ovary and uterus, rather than simply having a caloric effect. This has resulted in an increased interest in various oil seeds and in designing rumen inert fat sources that will deliver specific unsaturated FA to the lower gut for absorption. When cows fed fats containing mainly saturated and monounsaturated FA have been compared against a no supplemental fat control, the fat-supplemented cows generally have better conception rates. However, results comparing saturated and monounsaturated FA supplements in head-to-head comparisons with fat supplements containing a greater amount of polyunsaturated FA indicate that polyunsaturated FA-rich fats are more effective. Fat sources enriched in n-6 FA (e.g. most plant oils) or n-3 FA (e.g. linseed oil and fish oil) that deliver these fats to tissues beyond the rumen may therefore be the most effective ones to feed. Recently, the potential benefits of rumen-protected sources of conjugated linoleic acids (CLA) have also been of interest. Most data indicate that improved conception rates of fat-supplemented cows have been associated with an improved progesterone status of the cow by increasing the performance of the dominant follicle and corpus luteum and by helping the corpus luteum survive and continue to produce progesterone during the early days of pregnancy. These improvements have been proposed to be mediated via specific FA by: i) helping meet the animals' essential FA requirement in early lactation; ii) through the development of healthier ovarian follicles; iii) improving the quality of embryos produced; and iv) reducing embryonic mortality through suppression of uterine $PGF_{2\alpha}$. An additional and unique avenue through which CLA supplements may also assert their benefits on reproduction is via a reduction in milk fat synthesis, thereby sparing nutrients which may be used for other purposes by the animal. When considering individual FA and different fat supplements it is important to realise that different families of FA (e.g. n-6 vs. n-3 FA) most likely impact reproductive processes via different pathways. For example, n-3 FA have been shown in vitro and in vivo to have a suppressing effect on $PGF_{2\alpha}$ synthesis, whereas linoleic acid (n-6) promotes $PGF_{2\alpha}$ synthesis.

Conclusion. A variety of fat supplements have been tested for their effect on reproductive performance in lactating cows. Fats often improve pregnancy rates though there is large variability in responses observed; results, however, are rarely negative. Most data indicate that the observed improvements in reproductive parameters are independent of energy balance, while inconsistencies observed in the literature may be explained by variation in the availability of the specific fatty acids for incorporation into uterine tissues as a result of the extensive metabolism of dietary FA in the rumen. More research is needed to better identify the most effective fat sources and specific FA, whether from seeds and oils of plant origin, animal-derived fats, or commercially-available rumen-inert fat sources. The amount and type of supplement to be fed and the optimum window for supplementation will depend on the goals of the nutritional strategy employed and on the post-rumen delivery of specific FA from the supplement.

Why this continued interest in crossbreeding? Prospects for improving dairy reproduction and biology

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Among 50 respondents to a recent survey of U.S. dairy producers practicing crossbreeding, nearly all who bred Holstein females to sires of another breed cited a desire to reduce calving difficulty and a need to improve fertility, longevity and milk composition. Authors of the survey reported that respondents had achieved the improvements they sought.

One field trial in seven California commercial herds and three research trials in university herds are underway to investigate the benefits and disadvantages of combining breed additive and breed maternal influences with heterosis for dairy performance traits.

The trial in commercial herds revealed lower calving difficulty when first calf Holstein dams were bred to breeds of sire other than Holstein (e.g. Brown Swiss, Montebeliarde and Scandinavian Red) and lower stillbirth rate when multiparous Holstein dams were bred to these same non-Holstein sire breeds. The research trial of F_1 Jersey X Holstein sires bred to Holstein dams compared to Holstein sires bred to Holstein dams, reported results of lower calving difficulty scores and lower perinatal and pre-weaning mortality for the F_1sires.

An Australian study of 18 years of calving records of Holstein Friesian, Jersey and Jersey cross primiparous and multiparous calves revealed that male calves from Holstein Friesian dams had more dystocia and greater calf mortality than female calves whereas male and female Jersey-sired calves with crossbred or Holstein dams had less difference in dystocia and calf mortality between the sexes. There was no significant difference between sexes in dystocia in Jersey calves.

A full Holstein X Jersey diallele conducted in two university research herds has preliminary results on calving performance and heifer reproduction. Breed groups consisted of HH, HJ, JH and JJ with breed of sire listed first. Higher likelihood of problem births was found in HH,HJ and JH (in descending order) versus JJ calves. Based on progesterone level, average age at first ovulation was 324.6, 292.9, 296.8 and 285.8 days of age. Estimate of the breed additive effect was 34.9 days later for Holstein versus Jersey while the breed maternal effect was not statistically significant but heterosis was nearly statistically significant ($p<.10$). The field trial in California found that Normande X Holstein and Montebeliarde X Holstein first-calf crossbreds had fewer days to first breeding and higher first service conception rate than pure Holsteins and Scandinavian Red X Holstein crossbreds. Survival rates during first lactation favored all the crossbred groups over Holstein.

Immunological differences in calves from one of the herds in the Holstein X Jersey diallele were examined for the four genetic groups. Blood samples were analyzed for serum protein concentration and white blood cell counts. In general, JH calves had higher serum protein concentration and B-cell numbers compared to HH calves with HJ and JJ being intermediate. In related results from birth through weaning for the calves in the research trial from Holstein versus Jersey X Holstein sires mated to Holstein dams, calves from crossbred sires (i.e. ¾ H, ¼ J) had significantly higher total serum protein and serum IgG levels compared to pure Holstein calves.

As these trials progress, more data are accumulating on the advantages of crossbreeding for improving reproduction and fitness.

Heat detection – myths, magic and emerging science

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In the UK dairy industry most herds are being forced towards reducing fixed costs per litre of milk produced to match the downward pressure on milk prices. This has also to take account of the increasing costs of new technology and equipment needed for efficient milk production. The search for economies of scale has in many cases produced diseconomies of management with lack of staff time and skills, along with the demand to raise milk yields, causing a decline in fertility.
Fertility performance can be summarised as being firstly dependent on cows being served early enough, being served for long enough and then secondly the rate of pregnancies produced depends purely on the effect of heat detection and conception rate at any given oestrus. It is quite difficult to come up with a standard definition of heat detection and to be able to access data from commercial farms on how this parameter has changed over the years. Practice records simply measuring heat detection from return intervals has shown a progressive drop in performance over the last 20 years. Heat detection of returns has fallen from around 65% in the early 1980's to a level of around 40% now. This 20% change in heat detection has a profound effect on the efficiency with which pregnancies are produced and has reduced this efficiency figure from over 30% to below 20% now. Conception rates have dropped over the same period but in nowhere near the same proportions as with heat detection – 48% down to 38% over the same period of time.
As heat detection and conception rate are related variables it may in fact be that the main effect in practice is mostly due to heat detection and conception rate has simply to some extent followed due to inaccurate detection of cows for a successful service?
Heat detection is the most important factor in determining the number of pregnancies a herd produces in any period of time. It is far easier to manipulate than conception rate and is probably in some part helping to determine the conception rate.

Heat detection systems can be described under the following headings: -

1. Hormonal effects behind oestrus – hormone levels such as progesterone can be detected in blood or more conveniently in milk to indicate and precisely define oestrus activity. Also the effects of pheromone markers used by other cows (and possibly other animals e.g. dogs?)
2. Behavioural effects. The activities that are associated with oestrous activity can produce direct measurable markers – actual oestrus activity (mounting and riding etc) or indirect indicators such as increased movement.
3. Physiological effects. Temperature – most easily measured in milk – and also vaginal mucous conductivity and mucous arborisation patterns can be used.
4. Veterinary diagnosis from palpation or ultrasound scanning
5. Protocols to replace heat detection by synchronising the oestrous cycle sufficiently accurately enough to allow minimal observation for oestrus or preferably a fixed time insemination

Unfortunately under some of these headings there are restricting practical farm variables which profoundly affect the way the data is expressed or measured. For instance increased activity will not occur in cramped cubicles or on slippery concrete surfaces.

The other half of the herd – what's new in sperm science?

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Despite the title of this conference including only the female of the species, the use of the word 'fertility' necessarily implies some consideration of the provider of the male gamete. While the male is considered less problematic than the female, not all bulls are fertile. Some 5% of young dairy bulls entering AI are found to have unsatisfactory semen for processing which in many would be expected to affect fertility by natural service (NS). In the best young beef bulls after performance test, over 20% can have seriously deficient semen quality. NS also carries the risk of introduction of infectious infertility to the herd and is often a biosecurity blind spot.

With those caveats established, we shall now consider sperm physiology. Sperm are supplied for artificial insemination in excess numbers to allow for the lack of precision of quality assessment as a predictor of fertility. Even so, some bulls fail to reach acceptable fertility while others can be highly fertile at low sperm doses. For efficiency in processing and consistency in fertility outcome, improved practical methods for sperm evaluation are required.

In order to approach this problem in a logical way, it is necessary to understand the physiological processes in which sperm participate. Inseminated sperm face their first significant obstacle at the utero-tubal junction. Penetration depends upon quality of motility and probably also the ability of sperm to respond to the high bicarbonate environment of the oviduct with accelerated motility. The binding of sperm to isthmic oviduct epithelial cells (OEC's) creates a sperm reservoir. Identification of proteins involved in binding offers a method for assessment of sperm for binding ability. Evidence from other species suggests that OEC's influence bound sperm by inducing quiescence and inhibiting capacitation, while sperm alter OEC gene expression. OEC proteins affecting sperm may be useful in semen processing.

Ovulation-associated products in oviduct fluid encourage release of sperm from OEC's, with induction of capacitation and hyperactivated motility, the sperm now being adapted for binding to Zona Pellucida. There are prospects for more user-friendly assays of zona binding capacity. Having penetrated the zona, sperm are adapted to fuse with the oolemma. Up to this point, most of the stages in the sperm post-ejaculation life cycle involve surface modulation. Once the oocyte is penetrated, the final success of fertilisation depends upon the integrity of the DNA. Damage to DNA may be self-inflicted through apoptosis or may occur in response to the stresses applied in processing. Until recently, testing required expensive equipment or was very complex but new slide based methods offer the possibility of routine testing.

Processing always causes some damage to sperm and freezing mimics capacitation to a degree varying with the individual. A new assay for oxygen metabolism may give a means for assessment of these effects and so their influence on fertility.

From the above, it is not surprising that single-parameter quality testing fails to predict fertility with precision. Tools are now available for evaluation of all stages of sperm/female tract interaction and sperm integrity. These should improve efficiency and support necessarily low dose technologies such as sexed semen.

Body condition score and fertility - more than just a feeling

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Introduction
Although the scale of recording differs between countries, body condition score (BCS) is an internationally accepted, rapid, inexpensive and non-invasive method of estimating body energy reserves in dairy cows. Although limited by its subjectivity, it is independent of animal frame size and body weight. An up-to-date review of the literature on BCS, concentrating on its association with fertility, is presented.

Body condition score as a management tool Analyses of BCS profiles in dairy cows reveal a loss in early lactation up to 80 days post-calving after which replenishment of the lost body condition begins. This is a naturally evolved mammalian process, facilitating the use of stored energy reserves to produce milk. However, both level and rate of change in BCS at critical periods of the inter-calving interval have been associated with differences in animal performance across a range of production systems and countries. Although the effects on animal performance are sometimes non-linear or are parity dependent, in general, animals calving in greater BCS produce more milk of greater fat, and sometimes protein, concentration and superior reproductive performance. Body condition score has also been shown to influence somatic cell count. Most studies suggest that nadir BCS or BCS at the time of mating is more strongly related to reproductive performance than BCS at calving. Recent research indicates an increased odds of pregnancy to first service of 1.15 per standard deviation increase in BCS at first service. Furthermore, animals that lose more body condition in early lactation produce more milk of reduced fat and protein concentration, have lower somatic cell count, and inferior reproductive performance. Expressed on a per standard deviation basis, the odds of a successful pregnancy to first service was 0.79 and 0.76 per unit increase in BCS loss to first service or nadir, respectively. The effect on reproduction is probably linked with changes in both the somatotropic and gonadotropic hormone axes. Cows in low BCS at calving have delayed ovarian activity, a result of a reduced pulsatility of leutinising hormone, lower ovarian responsiveness to leutinising hormone, and ultimately through the closing down of follicular oestradiol production. However, despite the delay in ovulation associated with these factors, they are unlikely to be the BCS-mediated factors affecting pregnancy following a successful ovulation. Therefore other factors associated with the extent and severity of the negative energy balance must affect the ovary and pregnancy directly. One possibility is the smaller dominant follicles and corpus luteum in cows experiencing a greater negative energy balance. However, the extended period of uncoupling of the somatotropic axis in cases of extended negative energy balance, with the consequential lower production of IGF-1, is also a likely contributing factor. Another is the higher metabolic rate of high yielding cows with higher rates of clearance of progesterone by the liver.

Body condition score as a genetic tool Heritability is a statistic which quantifies the proportion of the observed variation among animals that can be attributed to differences in their genetic makeup. The heritability for BCS across different international populations range from 0.09 to 0.45, indicating that up to 45% of the variation in BCS among cows is due to genetics; heritability estimates for BCS change are lower (<0.19). Additionally, the reported strong genetic correlations between BCS at different stages of the inter-calving interval as well as between lactations suggest that BCS is influenced by similar genes. Consistent with the physiological data, increased BCS has been genetically associated with a shorter interval to the commencement of luteal activity, shorter interval to first service, greater submission rate, greater pregnancy rates, reduced number of services, and shorter inter-calving intervals. The favourable genetic correlations with fertility persist even after adjustment for phenotypic differences in milk yield among animals. However, there is also evidence that the genetic correlation with fertility changes with the stage of lactation that BCS is measured. Greater BCS loss in early lactation has also been associated with a greater number of days to first heat, first service, and the following calving. Fertility and survival are of low heritability and thus require larger numbers of daughter progeny to obtain accurate estimates of genetic merit for sires. However, the fact that BCS is visually assessed on all first lactation daughters of young test bulls, is moderately heritable and favourably correlated with fertility and survival, has resulted in BCS being used as a predictor trait for genetic merit for fertility in Irish and UK dairy cattle. The use of BCS as a predictor trait increases the accuracy of selection for fertility, the benefit of which is inversely related to the number of progeny per sire with actual fertility measures.

Body condition score as a decision support tool Increased herd size and difficulty in recruiting skilled labour necessitates a greater requirement for automation, in particular in relation to farm management. Recent research indicates the potential for automation of BCS using red laser light and digital cameras, the accuracy of which could be further augmented using multiple regression techniques incorporating other traits that can be automated such as milk yield and live-weight. Furthermore, recent research has also documented the possibility of experienced personal assessing cows for BCS using digital images on a subset of the herd (i.e., this service can be provided to many herds from one central location without any farm visits). Such information, in combination with other data sources may be subsequently used by farm managers in preferentially managing cows.

Integration of physiological mechanisms that influence fertility in dairy cows

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In dairy cows, successful reproduction is the result of a chain of events including resumption of oestrous cycles postpartum, development and ovulation of a healthy oocyte, fertilization, embryo development, implantation in the uterus, maintenance of pregnancy and parturition. Failure at any stage means failure of the whole process. The ovaries play a central role in the reproduction chain and compromised ovarian function, manifested as anoestrus or failure to conceive, is often a major cause of poor fertility. Ovarian function is regulated by a complex interaction of factors and feedback mechanisms at the cell (intra-follicle), organ (ovary) and animal (e.g. pituitary, pancreas, liver, adipose tissue) levels. At the follicle level, cell proliferation and development are controlled largely by endocrine and paracrine growth factors and nutrient supply. Collectively, these determine maturation and developmental competence of the oocyte. At the ovary level, developing follicles exhibit temporal changes in gonadotrophin receptors, local regulatory factors and their receptors, and steroidogenic enzymes, leading to recruitment and eventual selection of a dominant ovulatory follicle. At the animal level, reproductive cycles are controlled by gonadotrophins (LH and FSH) released from the pituitary gland in response to GnRH with feedback from ovarian hormones. A number of growth factors (e.g. insulin, IGF-1 and leptin) and metabolites (e.g. glucose, free fatty acids and ammonium) influence GnRH release from hypothalamic neurons. These growth factors affect other parts of the regulatory system by providing signals of metabolic status. The circulating concentrations of metabolites and hormones are determined by the balance between dietary inputs, level of production and body tissue reserves, relative to the animal's genetic potential. Genotype also influences ovarian responses to changes in metabolic signals.

Ovarian folliculogenesis can be divided into two phases. The early pre-antral stages of follicular development typically last 3 to 5 months and are largely independent of pituitary derived gonadotrophins. In contrast, the majority of antral-follicle development occurs within a single oestrous cycle and is dependent on gonadotrophic support. In addition, locally produced growth factors, which include members of the IGF and TGFβ superfamily of ligands, such as bone morphogenetic proteins (BMPs), are involved in follicular maturation.

Follicular growth is a developmental process during which the follicle progressively acquires a number of properties at specific times and in sequence, each of these being an essential prerequisite to develop to ovulation. The later stages of antral follicle development are characterized by two or three waves of follicular growth during each oestrous cycle. Each wave of follicle growth is characterized by recruitment of a group of follicles, which grow to approximately 6-8 mm in diameter. One follicle is then selected for continued growth and becomes dominant. The remaining follicles become atretic and regress. The precise mechanism for selection of dominant follicles remains to be fully elucidated, but does involve the action of gonadotrophins as well as locally produced factors. The presence of LH receptors on granulosa cells enables the dominant follicle to switch its gonadotrophic dependence from FSH to LH and attain dominance over other antral follicles, which remain FSH-dependent. Development of follicles also depends on interaction with a range of local and circulating growth factors. For example, increasing circulating insulin and IGF concentrations can increase both the number of gonadotrophin-responsive follicles and their quality.

Nutrition influences reproduction when diet is inadequate, excessive or imbalanced. For example, severe negative energy balance in postpartum dairy cows can impair ovarian function; diet can alter expression of growth factors linked to selection of the dominant follicle; diet composition can affect ooycte quality and blastocyst development. These responses are modulated by underlying genetics. Body fatness, itself determined by genetics, also alters responses to nutrition. Excess body fatness reduces food intake in dairy cows, leading to mobilisation of body fat, metabolic imbalances and impaired fertility; high levels of feeding in fat animals can result in hyperinsulinemia and reduced oocyte quality. We have shown that diets that increase plasma insulin concentrations stimulate earlier resumption of oestrous cycles postpartum. However, high concentrations of insulin can be detrimental to the developmental competence of oocytes, which is influenced also by supply of fatty acids at the systemic level and at the ovarian level. Insulin status is associated also with the incidence and characteristics of abnormal ovarian cycles. These changes can occur without significant variation in circulating gonadotrophin concentrations. This suggests that additional factors, such as peripheral metabolites, metabolic hormones and locally produced growth factors, may have a modulating role. Recent evidence has demonstrated that diet can impact at a variety of sites, and that ovarian responses to metabolic signals and nutrient profile vary according to stage of the reproductive cycle. This is supported by results of an initial study in our SEERAD-LINK-funded programme, where manipulation of circulating insulin and fatty acid concentrations at key stages of lactation increased pregnancy rate at 120 days from 27% to 60%.

In conclusion, the interactions between physiological mechanisms, genetics and nutrition can impact on ovarian function, including follicle growth and oocyte quality, with ensuing negative effects on early embryo development and foetal loss. Improved understanding of this multifactorial process enables nutrition to be matched to genotype, with a positive impact on pregnancy rate.

Acknowledgements: The work of the authors has been supported by Defra, SEERAD, LINK, BBSRC and NIH.

Fact and Fiction of Kiwi cow fertility: The New Zealand approach to breeding more fertile cows

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Introduction In seasonal production systems, good fertility is a fundamental part of the way in which farm systems operate. Feed requirements for production are matched to pasture supply on the majority of farms and in order to optimize pasture use a single concentrated seasonal calving pattern is required. Thus, achieving a 365-day calving interval is an important management aim. Targets to achieve a condensed calving include having half the herd calved within 14 days of the start of calving, 70% of the herd calved within the first 4 weeks of calving, and 95% of the herd calved within the first 8 weeks of calving.

Genetic Improvement A typical mating period lasts for 10-11 weeks, with 4-5 weeks of AI to generate replacements, followed by 6-weeks of natural mating. The short AI period and the tendency to cull, rather than carry-over empty cows has led to indirect selection for fertility in the New Zealand (NZ) population. Strain comparison studies (for example Moorepark in Ireland and the Dexcel Holstein-Friesian (HF) strain trial in NZ) have demonstrated the superiority of NZ strains of HF cattle; especially in their fertility. At the national population level the relationship between fertility and Holstein genetics is strong (e.g. Harris *et al*., 2006). The Jersey breed is even more fertile than NZ HFs, while crossbreeding of Jerseys and HFs leads to a further advantage due to heterosis. First cross HFxJersey cows have an advantage of 4.3% for being presented for mating in the first 21 days from the planned start of mating and 3.4% for calving in the first 42 days after the planned start of calving above the average of the parental breeds for first parity (Harris *et al*. 2006). HFxJersey currently comprise the greatest category of number of replacements born. One of the main reasons for the rise in popularity of crossbreds is that the national multi-breed genetic evaluations demonstrate an economic advantage of crossbreds over purebreds.

Management Management has a far greater impact on fertility than genetics, and farmers acknowledge that they have an enormous amount of managerial control over reproductive performance within their herd. A major emphasis is placed on matching the feed demand and supply curves for both milk production and conservation of cow body condition score (BCS). Industry targets of BCS 5.0 at calving (1-10 Scale) for 90% of multiparous cows and BCS 5.5 for primiparous heifers are strongly promoted. Recent field studies have also shown that pregnancy rate declines among cows less than BCS 4.0 at the start of mating. Accordingly, best-practice nutritional management is aimed at calving cows at BCS 5 and ensuring they lose no more than 1 BCS unit after calving. Extended postpartum anoestrus intervals are a particular problem for seasonal pasture-based production. One week before the start of the breeding period between 13% and 48% of cows were diagnosed as anovulatory anoestrus (Rhodes *et al*., 2001). A concern for the future is that replacement animals are kept from those that conceive after anoestrus treatment. This practice raises the possibility that there is indirect selection for animals with extended postpartum intervals. Astute managers will identify and differentially manage animals not meeting these targets. Modern practices include separating these 'at-risk' animals to reduce herd competition for feed, temporarily milking once daily to reduce energy demand and running bulls with this group.

Another emphasis is on managing the breeding event, with the overall aim of having 78% of cows reestablish pregnancy within the first 6 weeks of the seasonal breeding period. This can only be achieved when 90% of the herd inseminated within the first 21 days, return heats are detected with 90% efficiency and conception rates average 60%. Anoestrous rate and heat detection ability are the important drivers. Good inseminator technique is likely to be another positive factor. Trained technicians employed by the two main semen supply companies perform the majority of inseminations in NZ. The technicians' performance is continuously monitored, ensuring good insemination techniques. Heat detection has traditionally been good on NZ dairy farms, typically exceeding 90%. This is aided by cows being kept on pasture, which is a good surface to express oestrous behaviour in addition to large numbers of animals in oestrous at any one time (due to the seasonal calving system). However, increasing herd sizes and reliance on less-skilled labour is placing substantial challenges on maintaining a traditional high level of heat detection efficiency. Whilst overall fertility is still good in comparison to other countries, fertility is still a major issue for NZ dairy farmers, particularly getting sufficient animals pregnant to artificial insemination to generate replacements.

Conclusion Whilst conception rates may be high by international standards there are ongoing challenges to maintain and improve fertility of dairy cattle in New Zealand. This will include extension programmes to improve management and an ongoing focus on improving the genetic evaluation of fertility.

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Juvenile predictors of fertility

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Introduction Average fertility levels in the UK dairy population are low (pregnancy rate to first service 39.7%; Royal *et al.*, 2000a) and are similar throughout the developed world. Intense selection for milk yield over the last 30 years is, in part, responsible for the genetic and phenotypic decline in fertility. Many countries, including the UK, now publish and use genetic evaluations for female fertility. However, selection for fertility is difficult because traditional parameters, used in indices, have low heritability and can only be measured in the mature female. A potential solution would be the use of an indirect selection criterion that is measurable in young bulls, is heritable and is genetically correlated to the fertility of the bull's female progeny.

Juvenile predictors of fertility Research into juvenile predictors of fertility in cattle is somewhat limited and research to date can be broadly divided into reproductive hormones and metabolic traits.

Reproductive hormones Land (1973) first proposed that sex linked characters in the female are expressed in the male and that reproduction is controlled by the same gonadotrophic hormones in both sexes. In both male and female cattle, gonadotrophin releasing hormone (GnRH) is released by the hypothalamus which stimulates the anterior pituitary to synthesize and release follicle stimulating hormone (FSH) and luteinizing hormone (LH). Royal *et al*. (2000b) examined the LH response to a GnRH challenge in pre-pubertal (120-140 days) Holstein-Friesian heifers and found this to have a high heritability (heritability ± standard error; 0.51 ± 0.29, n = 206). Furthermore, the LH response to GnRH was genetically (-0.87 ± 0.25) and phenotypically (-0.17 ± 0.08) correlated to the interval to commencement of luteal activity postpartum (CLA) such that calves with a low LH response to GnRH challenge tended to have a long CLA as first lactation heifers (Royal *et al.*, unpublished).

Metabolic traits The length and severity of negative energy balance postpartum is unfavourably correlated (genetically and phenotypically) with interval to first ovulation (de Vries & Veerkamp, 2000). During this period concentrations of free fatty acids (FFA), glucose, growth hormone (GH), insulin, insulin like growth factor 1 (IGF-1) and other hormones, all of which have links with many aspects of reproduction, are altered. Concentrations of FFA, glucose, and insulin are moderately heritable (heritability range 0.11 to 0.27, n = 1498, at 9 months of age) in fasted pre-pubertal male Danish Holstein, Danish Jersey and Red Dane calves (Hayhurst *et al.*, 2007a) and in UK Holstein Friesian calves (heritability range 0.09 to 0.25, male n=256 and female n = 822, 4 months of age; Hayhurst *et al.*, 2007; submitted). In addition, heritability estimates for GH and IGF-1 in the UK Holstein are also moderate (heritability range 0.13 to 0.66, male n=256 and female n = 822, 4 months of age Hayhurst *et al.*, 2007b; Hayhurst *et al.*, 2007; submitted). Estimates of the genetic correlation of both male FFA and glucose concentrations with estimated breeding values for female fertility are negative and significant (-0.19 to -0.44, $P<0.01$, Hayhurst *et al.*, 2007) suggesting that on average, male calves with high glucose and FFA following an overnight fast at 9 months of age tend to produce female offspring with reduced fertility.

Conclusion It appears that genetic variation is sufficiently high in a number of parameters in calves to be useful as juvenile selection criteria. Additionally we have evidence to suggest that the LH response to GnRH and the concentration of FFA and glucose in calves are genetically related to female fertility and may therefore be of potential interest to dairy cattle selection programmes to improve female fertility. Further work is needed to confirm their relationship with female fertility and to determine their relationships with other traits of commercial importance to prevent unfavourable response to selection.

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Extended lactation: could it work for UK dairy farmers?

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Introduction Extended Lactation is a production system whereby dairy cows are managed for increased lactation persistency and rebred to calve at around eighteen months rather than twelve; the emphasis is on modest daily yield sustained over a long period rather than on peak yield. The potential benefits are avoidance of the health and reproduction problems increasingly associated with high peak milk yield. The potential costs are reduced per-annum milk yield and compromised milk quality. Both of these costs could be avoided if it were possible to increase lactation persistency, and modelling studies incorporating the use of rBST have predicted significant health and economic advantages to extended lactation (Allore and Erb, 2000; De Vries, 2006). For extended lactation to be successful under UK conditions, strategies for increasing persistency that do not include rBST are necessary. The introduction of automated milking systems has introduced the possibility of milking more frequently throughout lactation. Accordingly, we have examined the effect of frequent milking on lactation persistency in cows managed for eighteen-month extended lactations. Additional factors examined were nutrition and calving season. All three factors affected persistency, with the greatest improvement being attributable to milking thrice- rather than twice-daily. Milk quality was also maintained.

Materials and methods 24 Holstein:Friesian cows were used in a factorial design to study the effects of milking frequency (3X versus 2X), calving season (Spring *vs* Winter) and nutrition (High *vs* Low) on lactation characteristics during 18-month extended lactation cycles. Experimental treatments were applied from week 9 of lactation. Milking frequency treatments were applied on a half-udder basis to all cows. Low cows were fed a grass silage-based total mixed ration containing 15% crude protein during winter months and grazed pasture during summer supplemented with sugar-beet pulp during times of poor grass quality. In addition, in-parlour concentrate containing 18% crude protein was fed according to milk yield. High cows were fed identically except that they received an additional 3kg/d of concentrate. Cows were blocked onto treatment in groups according to calving date taking into account parity, body weight, body condition score and pre-treatment milk yield. Half-udder milk samples were collected at monthly intervals and analysed for processing quality by determination of casein number (casein as a proportion of total protein). Statistical analysis was performed using analysis of variance (Minitab Release11, Minitab Inc, State College, PA16801 USA). For analysis of lactation persistency, best-fit linear regression of weekly-averaged milk yields was performed from week 9 onwards.

Results Persistency was significantly improved by milking more frequently and non-significantly improved by supplementary feeding. Persistency was higher in Winter and Second than in Spring (Figure 1). Data shown are for the period prior to rebreeding. The beneficial effect of frequent milking (but not of season) persisted until week 20 of recurring pregnancy, at which point a negative effect of pregnancy became apparent. Extrapolations of actual persistency slopes were used to calculate the theoretical effect of improved persistency on lactation length in the absence of recurring pregnancy. Frequent milking increased lactation length from 68±3 to 102±8 weeks ($P<0.001$), whilst other treatments had no significant effect. Casein number decreased across the course of lactation, but this deterioration was prevented by the combination of frequent milking and nutritional supplementation (figure 2).

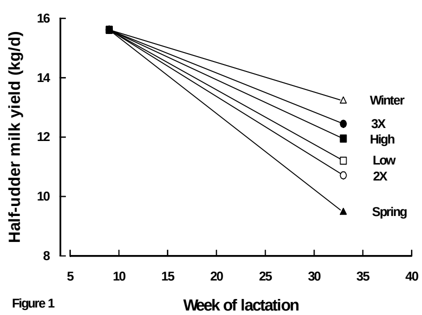

Figure 1

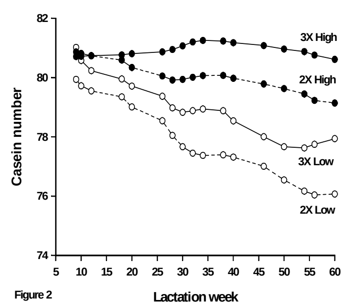

Figure 2

Conclusion By demonstrating that lactation persistency is plastic and can be improved by simple management interventions, the data lend support to the welfare and economic arguments in favour of extended lactation cycles.

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Expression profiles of genes regulating dairy cow fertility: recent findings, ongoing activities and future possibilities

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Dairy cow fertility at the phenotypic level is determined by the function of genes and their products and gene expression profiling is an important tool for addressing reproduction related questions. In cattle, gene expression profiling studies have, for example, targeted oocyte maturation (Dalbies-Tran and Mermillod, 2003, Vallee *et al.*, 2005), non-regressed and regressed corpus luteum tissue (Casey *et al.*, 2005), oviduct epithelial cell function (Bauersachs *et al.*, 2003, Bauersachs *et al.*, 2004), the endometrium during the oestrous cycle (Bauersachs *et al.*, 2005), pre-implantation embryonic development (El-Halawany *et al.*, 2004, Ushizawa *et al.*, 2004, Sirard *et al.*, 2005) and embryo-induced transcriptome changes of the endometrium in the pre- and peri-attachment period (Ishiwata *et al.*, 2003, Klein, 2006). Recently, in 2006, the EU integrated project SABRE (6th Framework Programme, Priority 5 Food Quality and Safety) was started, which aims to provide fundamental knowledge on the genomics and epigenetics of animal health, food safety and food quality traits of livestock species, together with strategies to deliver such technologies for use in selection. SABRE Work package 6 focuses on the genetic regulation of oestrus, folliculogenesis and embryo developmental competence. The transcriptomic research aims to identify genes that regulate critical factors for successful pregnancy at an optimum stage of lactation. Part of the work includes fine-mapping and further identification of a segregating QTL (BTA7) for conception rate in the Israelian Holstein population. The work generates fundamental knowledge on the genomics of important aspects of reproduction and fertility in dairy cattle and as such facilitates the development of successful breeding strategies for improved reproduction efficiency and a more sustainable livestock production.

The objective of this contribution is to give an overview of gene profiling studies on dairy cow fertility and to evaluate our current functional genomic knowledge about various processes underlying reproduction. Relevant ongoing research activities in SABRE are presented and the pro and cons of gene profiling studies for understanding female fertility are discussed.

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Are we making the most of current indicators? Experience from National Recording Schemes

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Introduction Research conducted by Teagasc at Moorepark utilising strains of Holstein Friesian cows in the late 1990's established that dairy cow fertility under Irish farming conditions had a large genetic component. Substantial differences in empty rates at the end of the breeding season indicated that dairy cow fertility on many Irish farms had been dramatically reduced through breeding decisions taken over a period of some twenty years. Recent evidence, derived from the genetic evaluations of bulls selected for use in artificial insemination confirms this trend (Figure 1).

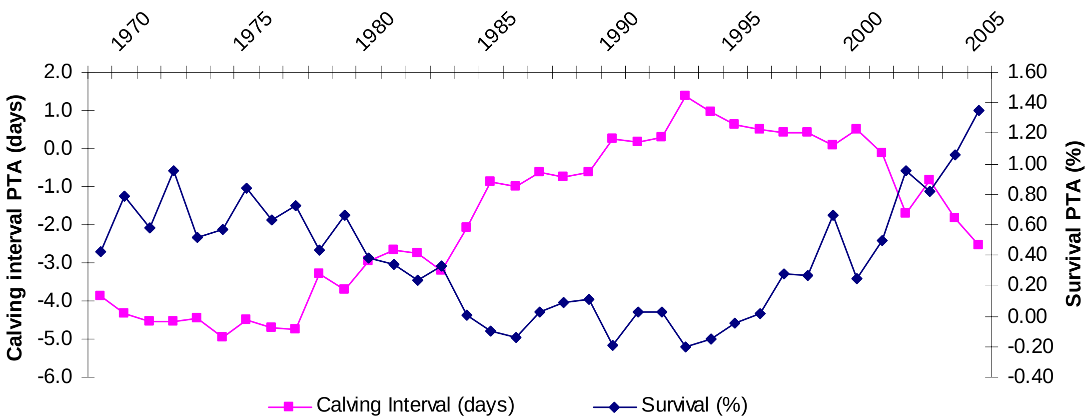

Figure 1 Average breeding values of Holstein Friesian bulls registered for use in artificial insemination by year of birth.

Over a twenty year period starting in the early 1970's the predicted transmitting ability for calving interval of bulls selected for use in AI increased by some six days while that for survival (from one lactation to the next) declined by some 1%. In 1998 the Irish Cattle Breeding Federation (ICBF) commenced operation with the objective of increasing the rate of genetic gain on Irish cattle farms. It was given responsibility for establishing a cattle breeding database, upgrading the genetic evaluation systems and optimising breeding scheme design for both the beef and dairy breeding industries. This paper reports on the methods employed by ICBF to address the decline in fertility of Irish dairy cattle.

Methods A cattle breeding database was established by consolidating the data previously held in a range of systems servicing milk recording, herd book registration, genetic evaluations, official calf registrations and cattle movements. The database replaced some 35 separate systems that were involved in the provision of information services to dairy and beef farmers. A modified "animal events" system for collecting data from farms was introduced to remove duplication of data collection by farmers while, at the same time, providing the data required to support official calf registration, pedigree registration, milk recording, and genetic evaluation of both dairy and beef cattle. Genetic evaluation systems for all traits relevant to dairy (and beef) production in Ireland were reviewed in the light of a profit based breeding objective appropriate to the physical and economic environment of Irish cattle farming. Calving intervals and survival from one lactation to the next were used as indicator traits for fertility. Through a combination of Interbull services and custom pair wise collaborations, international genetic evaluations for fertility traits have been developed. Research identified the optimal breeding scheme design for Ireland and formed the basis for the introduction of a shared progeny test scheme known as G€N€ IR€LAND®.

Results The cattle breeding database became operational on a limited scale in 2002 and is now fully operational. The volume of data available for genetic evaluation purposes has increased orders of magnitude (e.g. 20,000 to 500,000 records of calving difficulty per year). Performance data on non-pedigree cattle with known sires has also increased dramatically. The across breed EBI (economic breeding index) has become the main criteria for breeding decisions in dairy cattle. Sub-indexes are used to distinguish the trait groups that contribute to profitability. Calving interval (on the first 3 parities) and survival (first 3 parities and lifespan as a predictor) are the current proxy's for fertility and account for some 37% of the emphasis in the EBI. G€N€ IR€LAND® is now in its third year of operation and in 2007 involved bulls from three AI companies. Figure 1 shows the genetic trend of the bulls selected for use in AI. The last few years have seen a dramatic change in both calving interval and survival.

Conclusion Genetic trends in the fertility of a commercial population of dairy cattle can be measured and their direction determined through a combination of data collection, genetic evaluation and breeding scheme design. In a period of nine years the Irish dairy breeding industry has established the infrastructure required to reverse an undesirable trend in the fertility of the commercial dairy cattle population using calving interval and survival as indicator traits for fertility.

The effect of changing dietary amino acid composition on plasma metabolite and hormone concentrations and on oocyte quality in Holstein heifers

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Introduction We have previously shown (Adamiak *et al.* 2005, 2006) that the quality of oocytes recovered by ovum pick up (OPU) from heifers is influenced by the composition of the diet and the body condition of the donor. In particular, increasing the amount of starch in the diet is associated both with decreased development to the blastocyst stage and with high plasma insulin concentrations; this effect was dependent on body condition. Branched chain amino acids, particularly leucine, stimulate insulin release from a variety of pancreatic models. Therefore manipulation of plasma insulin concentrations and thereby the developmental potential of the oocyte, by increasing the supply of dietary leucine could provide an alternative to feeding diets containing large proportions of starch. The present study therefore investigated the effects on oocyte quality of manipulating diet composition to increase plasma leucine concentrations. The diets were chosen so that the interactive effects of dietary leucine and carbohydrate provision (starch- or fibre-based diets) could be assessed.

Materials and methods Holstein heifers weighing 522 (sd, 37) kg and with a condition score of 2.2 (sd, 0.52) units were assigned (8 per treatment) to either a fibre- (F, 355 g neutral detergent fibre (NDF) and 149 g starch / kg dry matter (DM)) or starch-based (S, 148 g NDF and 420 g starch /kg DM) concentrate diet containing either low (L, 8.3 g/kgDM) or high (H, 10.3 g/kg DM) dietary leucine giving a 2x2 factorial arrangement of treatments (FL, FH, SL, SH). Oestrus was synchronised by CIDR and OPU began 7 days after CIDR removal. Each heifer underwent 6 sessions of OPU (2 per week for 3 weeks). Oocytes recovered were matured, fertilised and cultured in synthetic oviductal fluid *in vitro* to the blastocyst stage. Individual animal identity of oocytes was maintained throughout. Blood samples were obtained from each heifer before and 1.5h after feeding, once weekly for 3 weeks. Plasma hormone concentrations were determined by radio-immunoassay and free amino acids by HPLC of o-phthaldialdedyde derivatives. Plasma hormone and amino acid concentrations were analysed by repeated measures ANOVA within Genstat 6 employing a 2x2 factorial treatment design. *In vitro* culture data were analysed using a generalised linear model with binomial distribution and logit link function also within Genstat.

Results Feeding diets containing high leucine resulted in higher plasma leucine (LvH; 13.1v16.4 μg/ml; SED, 0.62; $P<0.001$) and tyrosine (LvH; 6.1 v 6.6; SED, 0.26; $P=0.035$) concentrations. Post-feeding insulin and glucagon concentrations were greater ($P<0.001$) than before feeding but there were no interactions between sample time and treatment. Feeding starch increased mean plasma insulin concentrations (Table 1) whereas increasing dietary leucine increased mean plasma glucagon concentrations and the insulin:glucagon ratio.

Table 1 Plasma insulin and glucagon concentrations (ng/ml; mean of pre- and post-feeding samples)

Diet	FL	FH	SL	SH	SED	F v S	L V H
Insulin	1.48	1.39	1.89	1.81	0.255	P=0.028	NS
Glucagon	0.13	0.16	0.13	0.16	0.017	NS	P=0.011
Insulin:glucagon	11.9	9.3	14.8	11.2	1.78	NS	P=0.021

There were no significant interactions between dietary carbohydrate and leucine. SED for 48 observations.

There were no differences between treatments in the numbers or quality of oocytes recovered by OPU. Oocyte cleavage (mean ± sem; FvS, 0.57± 0.037v 0.52±0.032) was greater for fibre-based diets but the difference was not significant. There was an interaction ($P=0.033$) between dietary carbohydrate and leucine for blastocyst development (from cleaved oocytes) such that leucine had no effect on fibre-based diets (FLvFH; 0.29±0.056 v 0.31±0.049) but improved blastocyst development on starch-based diets (SLvSH, 0.18±0.045 v 0.30±0.052). Overall, blastocyst development from matured oocytes tended to be greater when the fibre-based diet was fed (FvS; 0.19±0.026 v 0.12±0.020; $P=0.10$).

Conclusions Feeding starch-based diets increased plasma insulin concentrations and tended to adversely affect oocyte development. Increasing dietary leucine increased plasma leucine concentrations but increased plasma glucagon rather than insulin concentrations. The positive effect of dietary leucine on embryo development when starch-based diets were fed may be related to reductions in insulin:glucagon ratio.

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Study funded by Defra. SAC receives financial support from SEERAD

Mastitis induction of delayed ovulation and its relation to follicular functions and luteinizing hormone concentrations in lactating cows

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Introduction Epidemiological studies have documented attenuated reproductive performance in cows having mastitis. Decreased conception and increased days to first AI as well as days open have been shown to be affected by both clinical and sub-clinical mastitis events, either before or after AI, induced by either gram-negative or gram-positive pathogens (Santos *et al*., 2004; Shrick *et al*., 2001). However, the actual reproductive mechanisms disrupted by pathogenic stress remain unclear. Exposure of cows to endotoxin (LPS) during the follicular phase reduced pulsatile luteinizing hormone (LH) concentrations and induced delayed ovulation (Suzuki *et al*., 2001). We showed (Lavon *et al*., 2004) that LPS administered at the onset of estrus induced delayed ovulation in 33% of cows. In naturally occurring mastitic cows, resumption of cyclicity post-partum has been shown to be delayed compared with that in healthy cows (Huszenicza *et al*., 2005). We recently found (Lavon *et al*., 2006), in cows synchronized with two doses of $PGF_{2\alpha}$, that 26% ($P<0.03$) of cows with naturally occurring clinical or sub-clinical mastitis, compared with only 4% of healthy cows, manifested delayed ovulation, or did not ovulate. Delayed ovulation was reflected in a delay in the rise of progesterone. In addition, the duration of dominance of the preovulatory follicle tended to be longer, and the number of follicles during the cycle was 20% lower in mastitic than in healthy cows. The aim of the present study was to examine the effects of naturally occurring mastitis on the functioning of the preovulatory follicle and on gonadotropin secretion.

Materials and methods Cyclic lactating Holstein cows were diagnosed for mastitis type by somatic cell counts and bacteriological examination at the quarter level. Estrus-synchronized cows (95 days post-partum) were observed for estrus and ovulation times were determined by ultrasonography. Blood samples were taken for preovulatory estradiol and LH surge from 11 healthy cows, 10 cows that exhibited clinical mastitis events 33 days earlier, and 12 cows that exhibited sub-clinical, chronic mastitis. For pulsatile LH secretion, a 6-h window of frequent samples was taken from 5 healthy, 5 clinical mastitis, and 6 sub-clinical mastitis cows during the follicular phase 36 h after $PGF_{2\alpha}$. Hormones were determined by validated RIA or ELISA. Data were analyzed by ANOVA using the GLM of SAS. Means and SE are presented.

Results Cows with clinical or sub-clinical mastitis, which manifested an extended estrus-to-ovulation interval, exhibited lower concentrations of estradiol at estrus, compared with healthy cows or mastitic cows, which manifested normal estrus-to-ovulation intervals (3.3±0.4, 5.8±0.5, and 5.9±0.8 pg/ml, respectively; $P<0.01$). Healthy cows or mastitic cows (clinical or sub-clinical) with normal intervals, exhibited normal peaks of LH surges at the expected time relative to onset of estrus (6.9±0.6 ng/ml, 4.6±0.6 h). In contrast, mastitic cows with delayed ovulation exhibited a delayed LH surge (10.5±3 h relative to onset of estrus, $P<0.01$) with either low or normal LH surge, or no LH surge was evident. Regarding pulsatile LH, no statistical differences were detected between healthy, clinical, or sub-clinical mastitic cows in their mean, pulse amplitude, and pulse frequency of LH concentrations (1.3±0.1 ng/ml, 0.73±0.1 ng/ml, 4.9±0.2 pulses/6 h, respectively).

Conclusions Results indicate that short-term clinical mastitis events or sub-clinical, chronical mastitis induced, in about 30% of the cows, a delayed ovulation syndrome associated with low estradiol concentrations. However, this was not associated with altered pulsatile LH secretion during the follicular phase, suggesting a direct effect of mastitis on steroidogenesis. Delayed ovulation, which was associated with low or delayed LH surge, could be related to low preovulatory estradiol levels. The above could explain, in part, mastitis-induction of low fertility in dairy cows.

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Distribution of follicle numbers in dairy cows aged from 2 to 11 years

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Introduction The number of antral follicles in cattle is highly repeatable in individuals, while being highly variable between animals (Burns et al., 2005; Ireland et al., 2007). Furthermore, a high antral follicle number is associated with a greater number of transferable embryos following superovulation (Singh et al, 2004; Ireland et al, 2007). However, the relation between age of the cow and follicle numbers has not been investigated. Furthermore, the ability to phenotypically classify cows with respect to follicle number based on a single rather than multiple ultrasound examinations would be desirable to rapidly identify cattle with high responsiveness to superovulation. The aim of this study was to investigate the accuracy of a single ultrasonographic examination, performed on a random day of the oestrous cycle, and to assess the variability in the number of follicles in dairy cows of different ages.

Materials and methods Transrectal ultrasonography was performed on 116 dairy cows, aged from 2.6 years to 10.8 years, to assess the number of ovarian follicles ≥3 mm in diameter on a random day of the oestrous cycle. Based on this scan, a subset of animals was selected as follows: cows with less than 15 antral follicles ≥3 mm in diameter (Low group n= 8) and those with more than 30 follicles ≥3 mm in diameter (High group, n=10). Both groups were synchronized with 2 injections of PGF2α (Estrumate®, Loughrea, Co., Galway, Ireland) 11 days apart and the number of follicles ≥ 3 mm in diameter was counted daily during an entire oestrous cycle. The correlations between follicle number and animal age and between a single ultrasound examination and the maximal number of follicles during follicular waves were analyzed using ANOVA.

Results Amongst the 116 animals**,** the number of antral follicles ≥3mm in diameter per animal ranged from 4 to 44, with a mean (±SEM) of 17.99±0.78 (Fig.1). Cow age was not related to the number of follicles detected on a random day of the oestrous cycle ($R^2 = 0.0086$; P=0.31). Amongst the subsets of animals, the mean (±SEM) number of follicles was 35.9±1.50 in the High group and 8.13±0.71 in the Low group. No difference (P=0.89) was detected between the two groups in mean age (High=5.28±0.43 years, Low=5.38±0.62 years). Numbers of follicles at the initial random scan were highly correlated with the maximal number of follicles observed during follicular waves of a complete oestrous cycle (R^2 =0.7756; P <0.0001).

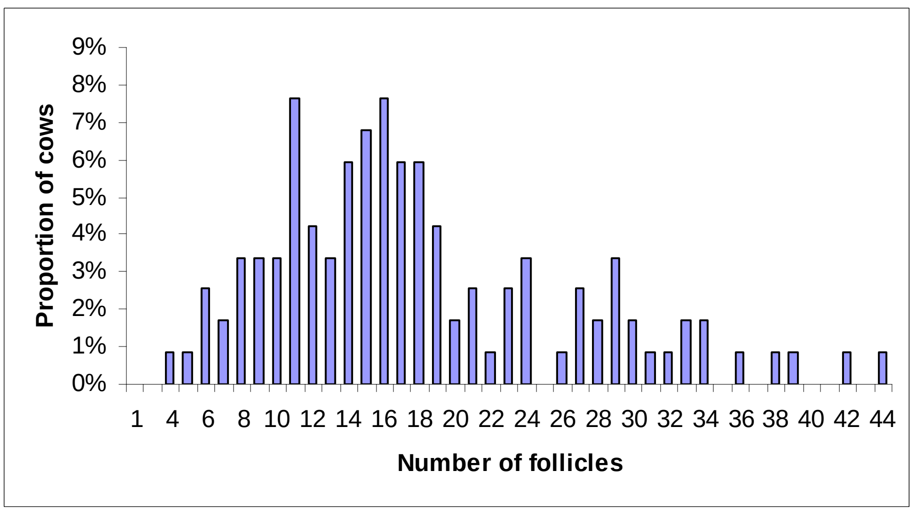

Figure 1: Frequency distribution of the number of follicles ≥3mm in diameter on a random day of the oestrous cycle (n = 116).

Conclusion The number of antral follicles in the ovaries of dairy cows is highly variable among cattle but apparently not linked with cow age. Cows with consistently high or low numbers of follicles can be reliably identified through transrectal ultrasonography performed on a single random day of the oestrous cycle

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Arcanobacterium pyogenes pyolysin stimulates bovine endometrial cell prostaglandin secretion

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Introduction Uterine disease associated with infection by *Arcanobacterium pyogenes* and *Escherichia coli* affects 40% of cattle after parturition and causes considerable infertility. The most severe pathogen isolated from the postpartum uterus is *A. pyogenes*, which secretes a haemolytic exotoxin pyolysin (PLO), whilst *E. coli* has an endotoxin, lipopolysaccharide (LPS). The effect of *E. coli* and LPS on the reproductive function of cattle has been extensively investigated, but the role of *A. pyogenes* is less clear. Endometrial epithelial and stromal cells are the first line of defence, secreting prostaglandin $F_{2\alpha}$ (PGF) and prostaglandin E_2 (PGE), which have principally endocrine and immune roles, respectively. Modulation of prostaglandin secretion from the endometrium may disrupt ovarian cycles and cause infertility. The objective of the present study was to test the hypothesis that *A. pyogenes* modulates the function of endometrial cells, and investigate the effects of PLO on prostaglandin production by endometrial cells *in vitro*.

Materials and methods Endometrial epithelial and stromal cells were enzymatically isolated from the uterus collected from heifers at the slaughterhouse and cultured for 10 days until confluent. A bacteria-free filtrate (BFF) was cultivated by growing *A. pyogenes* in a brain-heart infusion broth for 48 h, which was filter sterilized and stored at -80^0C. *A. pyogenes* was isolated from the BFF, were heat-killed (HKAP) for 15 min at 68 ^{0}C and diluted to yield a working concentration of 1.6 x 10^{10} CFU/ml. The PLO toxin was cultivated from a plasmid encoding the mature form of PLO. Epithelial and stromal cells were treated for 24 h with increasing concentrations of HKAP (10 > 10^5), BFF (equivalent to 0.81 CFU/ml) and 0.3 ng/ml PLO alone. Supernatants were collected, and prostaglandins measured by RIA.

Results Prostaglandin production from epithelial and stromal cells treated with HKAP remained basal and no significant differences were observed when compared to controls. Epithelial cells treated with BFF when compared with controls, secreted more PGE (30 ± 15 vs. 8 ± 5 ng/ml; P<0.01) then PGF (20 ± 4 ng/ml vs. 9 ± 1 ng/ml; P<0.05). Stromal cells treated with BFF also secreted more PGE (26 ± 6 ng/ml vs. 16 ± 2 ng/ml; P>0.05) when compared with controls but produced little PGF. Epithelial cells treated with PLO, secreted more PGE (74 ± 17 vs. 38 ± 3 ng/ml P<0.05) then PGF (27 ± 3 vs. 10 ± 0.5 ng/ml, P<0.05), and stromal cells treated with PLO, secreted less PGE (34 ± 1 vs. 49 ± 10 ng/ml P<0.05) and little PGF, compared to controls. The viability of epithelial and stromal cells were also analysed for cells treated in a dose manner for 24 h with PLO alone. The results showed that (Table 1) the viability of stroma had decreased at concentrations 0.1 and 0.3ng/ml, in addition stroma treated at higher concentrations were undetectable. However, the viability of epithelial cells treated with PLO was comparable to controls at the lower concentrations, with decreases observed at the higher concentration of 1 and 3ng/ml of PLO.

Cell numbers	***Epithelia***	***Stroma***
Control	*24.1 x 10^6*	*19.1 x 10^6*
PLO (ng/ml)		
0.1	17.2 x 10^6	5.4 x 10^6
0.3	21.8 x 10^6	1 x 10^6
1	10.3 x 10^6	ND
3	5.23 x 10^6	ND
LPS	*21.8 x 10^6*	*19.8 x 10^6*

Conclusion In conclusion, heat-killed *A. pyogenes* did not affect endometrial function but a bacteria-free filtrate or the exotoxin PLO modulated prostaglandin secretion by epithelial and stromal cells, supporting the concept that endometrial cells have an important role in uterine immunity. Analysis of cell survival also supported the results observed for prostaglandin production by epithelial and stromal cells, confirming that notion that the toxin of *A. pyogenes* can cause severe damage to the bovine endometrium.

Effect of progesterone supplementation post conception on pregnancy rate in cattle

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Introduction Progesterone is the essential hormone for the establishment and maintenance of pregnancy in mammals (Spencer and Bazer, 2002). In cattle, low concentrations of progesterone in the first days of pregnancy (Day 3-8) have been associated with smaller embryos, which produce less interferon tau around the critical period of maternal recognition of pregnancy (Day 16) and, as a consequence, are less likely to maintain a pregnancy (Mann and Lamming, 2001; Stronge *et al*., 2005). Progesterone supplementation, by means of progesterone injections, intravaginal devices or induction of accessory corpora lutea (CL) all lead to an increase in circulating concentrations of progesterone but reported effects of such supplementation on pregnancy rate have been variable (Cleeff *et al*., 1996; Starbuck *et al*., 1999). This study was carried out to examine the effect of elevating progesterone concentrations for several days during the first week post conception on embryo survival.

Materials and methods Commercial beef cross heifers (n=197, approx. 2-years old) were synchronised using two injections of a prostaglandin $F_{2\alpha}$ analogue (PG, Prolsolvin, Intervet Ireland Ltd) given 9 days apart. The heifers were checked for signs of oestrus 4 times daily commencing 24 h after the second PG injection and were inseminated 12-18 h after the first signs of oestrus (= Day 0). Inseminated heifers were randomly assigned to 1 of 3 groups: (i) Control, n=69; (ii) Progesterone supplementation by means of a Controlled Internal Drug Device (CIDR 1.9g, Pfizer UK) from Day 3 until Day 6.5, n=64; (iii) Progesterone supplementation from Day 4.5 until Day 8, n=64. This treatment has been shown to result in an immediate 1 to 2 ng/ml elevation in progesterone concentration (Carter *et al*. unpublished). Pregnancy was determined at slaughter at approximately Day 25. Conceptus length and weight were recorded and the corpora lutea of all pregnant animals were weighed. Data were analyzed by Chi-square analysis or Student’s t test, where appropriate.

Results Supplementation with exogenous progesterone as outlined in the material and methods above did not significantly affect pregnancy rate compared with the controls (Table 1). There was no significant difference in mean CL weight or conceptus length or weight between the 3 groups of beef heifers.

Table 1 Effect of exogenous progesterone (P4) post-insemination on pregnancy rate, CL weight and conceptus characteristics (mean ± s.e.) at Day 25

Treatment	No. Animals	Pregnant (%)	CL weight, g	Conceptus weight, g	Conceptus length,cm
Control	69	29 (42.0)	5.84 ± 0.19	0.49 ± 0.06	29.48 ± 2.27
P4 Day 3 to 6.5	64	22 (34.4)	6.03 ± 0.15	0.55 ± 0.06	30.24 ± 3.00
P4 Day 4.5 to 8	64	31 (48.4)	5.42 ± 0.16	0.55 ± 0.05	31.51 ± 1.99

Conclusion Under the conditions of this study, supplementation of progesterone concentrations via CIDR insertion between Days 3.5 and 8 did not alter pregnancy rate. In addition, there was no effect on either CL weight or conceptus characteristics in pregnant animals.

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Adult dairy cow performance is influenced by her maternal intrauterine environment during development

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Introduction A substantial amount of evidence, based primarily on epidemiological studies of human data, suggests that perturbations during fetal life are associated with hypertension, vascular dysfunction, dyslipidaemia, and insulin resistance in adulthood (Barker, 1995). Few studies have attempted to quantify the influence of maternal environment on subsequent progeny performance in dairy cattle. Pryce et al. (2002) reported no significant effect of dam parity or milk production, dry matter intake or BCS in the first or second 13 weeks post-partum on subsequent female progeny reproductive performance. The objective of this study was to quantify the maternal variance for performance indicators in first, second and third lactation dairy females using a large national database.

Materials and methods National data on Holstein-Friesian dairy cows from the Irish Cattle Breeding Federation database were obtained. Non-singleton animals, animals born from embryo transfer, and animals with no known sire or dam or from paternal half-sib groups of less than 5 were removed; maternal grandsires with less than 5 granddaughters with records were also removed. Dam lineage was determined for each animal by tracing back the pedigree to the founder female; lines with less than three females with records were removed. Following all edits 188,144 parity 1 to 3 lactation records from 80,881 animals remained. (Co)variance components were estimated for 305-day milk yield, somatic cell score (SCS; i.e., Log_eSCC), calving interval, and survival separately within parity using a linear mixed model in ASREML (Gilmour et al., 2007); (co)variance components were also estimated for age at first calving. Fixed effects differed by trait analyzed, but for age at first calving were herd-year of calving, year of birth-month of birth interaction, heterosis, recombination and Holstein percent; while for all other traits herd-year of calving, year of calving-month of calving interaction, heterosis, recombination, Holstein percent, age at calving and whether calving difficulty was experienced at calving were included in the model. Random effects included in the model were an additive direct genetic, cytoplasmic, remaining maternal (nuclear) genetic, permanent environmental effect of the dam, and a residual component. A covariance component was also estimated between the additive direct and maternal components where possible. Because calves in Ireland are generally separated at birth from dairy cows, a significant maternal variance (with all other random effects in the model) was assumed to suggest the presence of intrauterine effects on progeny performance.

Results and discussion All additive direct genetic effects were significant and direct heritability estimates varied from 0.01 (survival to parity 3) to 0.34 (milk yield in parity 2 animals). The maternal component was only significant for milk yield in first and third parity, survival to second lactation and SCS in first parity. Despite its statistical significance, however, the maternal variance accounted for less than 1% of the phenotypic variation. Furthermore, the coefficient of variation accounted for by the maternal component was less the 3%. The significance of the maternal genetic effect (excluding cytoplasmic effects) and covariance with the direct additive genetic effect for milk yield is in contrast to previous results from US Holsteins (Schutz et al., 1992) where no significant (co)variance was reported. However, our data suggest that intrauterine conditions during gestation may have repercussions for subsequent progeny lactation performance. The significance of the maternal variance for survival to second lactation remained even when first lactation milk yield and SCS were included as fixed effects in the model; this implies that intrauterine conditions affect survival over and above the intrauterine effect on progeny milk yield and SCS. Maternal lineage had a significant effect only on milk yield in third parity animals and SCS in second parity animals explaining less than 1% of the phenotypic variation in both traits. The correlation between the direct additive genetic component and the maternal component where estimated were all negative thereby indicating a difficulty in improving both components simultaneously; this may be an artifact of the change in allelic frequency due to simultaneous selection on both components over time.

Conclusion Significant maternal variation was observed in milk production, SCS and survival to second lactation. Because of the dairy farming system operated in Ireland where the calf is generally removed immediately post-calving from the cow this suggests that pre-natal factors (i.e., intrauterine condition) affect the subsequent performance of the offspring. Although the effect is small and unlikely to bias greatly genetic evaluations of sires it does suggest that adverse conditions experienced by the dam during pregnancy may have repercussions for offspring performance.

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Effects of dry period duration and dietary energy density on transition period metabolic status and postpartum ovarian function in dairy cows

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Introduction The metabolic and endocrine milieu that ensues during negative energy balance (NEB) is antagonistic to resumption of ovulatory ovarian activity, resulting in anoestrus and reduced conception rates. Reducing the length of the dry period (DP) has been shown to improve early lactation energy balance and fertility indices (Gumen et al., 2005). This study was carried out to determine if dry period duration and dietary energy density influenced metabolic status and indicators of reproductive efficiency.

Materials and methods Forty mature Holstein-Friesian cows were used in a completely randomized block design with a 2 x 2 factorial arrangement of treatments. Cows were assigned to one of two dry period treatments (standard dry period (SDP) OR no planned dry period (NDP)) and one of two nutritional treatments (standard energy TMR (STMR) OR high energy TMR (HTMR)) Four cows were removed from this experiment due to health problems unrelated to the study. Blood samples were collected from the coccygeal vessels and analysed for insulin, glucose and nonesterified fatty acids (NEFA) concentrations. Ovarian follicular activity was monitored via transrectal ultrasonography beginning on Day 8-10 post partum (PP) and continuing until first PP ovulation. Circulating oestradiol was measured in daily blood samples collected during the first PP follicular wave. Data were analyzed using the MIXED procedure of SAS, using repeated measures where appropriate. Conception and pregnancy rate data were analysed using Fisher's exact test.

Results The insulin, glucose and NEFA results (Table 1) indicate that periparturient metabolic status was improved in response to omitting the DP (SDP vs. NDP), and also in response to feeding a higher energy TMR (STMR vs. HTMR). Cows assigned to NDP had an earlier resumption of cyclicity than cows assigned to SDP (16.8 ± 2.5 vs. 24.8 ± 2.6 days PP, P = 0.002), but dietary energy density did not have a significant effect (20.9 ± 2.5 vs. 20.7 ± 2.7 days PP; STMR and HTMR, respectively; P = 0.9). For cows that had an ovulatory first PP follicle wave, maximum follicle diameter (16.8 ± 1.3 vs. 15.1 ± 1.1; P = 0.16) and peak circulating oestradiol concentrations (3.7 ± 0.5 vs. 3.2 ± 0.4 pg/ml; P = 0.2) were not different between SDP and NDP, respectively. Similarly, maximum follicle diameter (15.8 ± 1.3 vs. 16.1 ± 1.1; P = 0.8) and peak circulating oestradiol concentrations (3.3 ± 0.5 vs. 3.7 ± 0.4 pg/ml; P = 0.4) were not different between STMR and HTMR, respectively. Omitting the DP had no effect (P>0.05) on conception rate to first service, but conception rate tended (P = 0.08) to be higher for cows fed STMR compared to HTMR (Table 1). Neither DP duration nor dietary energy density had an effect (P>0.05) on other aspects of reproductive performance (Table 1).

Table 1 Summary of the metabolic status and indices of reproductive performance

	Dry Period (DP)		Nutrition Level (NL)		SEM	P - value		
Metabolic status Day -21 to -1	SDP	NDP	STMR	HTMR		DP	NL	DP x NL
Insulin (μIU/mL)	5.30	8.31	6.16	7.46	0.95	0.032	0.34	0.09
Glucose (mmol/L)	3.42	3.60	3.44	3.57	0.05	0.015	0.078	0.022
NEFA (mmol/L)	0.11	0.05	0.09	0.07	0.02	0.018	0.49	0.24
Metabolic status Day 0 to 28								
Insulin (μIU/mL)	3.92	7.36	4.54	6.76	0.58	<0.001	0.006	0.9
Glucose (mmol/L)	3.33	3.69	3.40	3.62	0.05	<0.001	0.001	0.42
NEFA (mmol/L)	0.30	0.20	0.28	0.22	0.02	0.007	0.13	<0.001
Number of cows	18	18	17	19				
Calving to service interval (days)	79	83	77	85	7.1	0.6	0.3	0.8
Conception rate to 1st service (%)	33.3	38.9	52.9	21.1		0.9	0.08	
Calv. to conception interval (days)	123	112	114	120	11.1	0.4	0.6	0.7
Overall pregnancy rate (%)	66.7	83.3	82.4	68.4		0.4	0.5	

Conclusions Omitting the DP or feeding a high energy TMR resulted in superior metabolic status. Feeding a high energy TMR had no effect on onset of cyclicity, but tended to have a negative effect on conception rate to first service. This negative effect may be due to the greater clearance of steroid hormones associated with greater intakes. Further work is warranted on manipulation of DP duration as a means to improve metabolic status and reproduction.

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The effect of crossbreeding on reproduction parameters and milk production of Iranian native cattle.

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Introduction. Crossbreeding is used widely for many livestock species because improvement of milk and fat yield, fertility, calf survival, health and live weight by introducing favorable genes from other breeds and maintaining the gene interaction that cause heterosis (Carraviello et al 2002). The success of a crossbreeding program will depend on the quality of breeds chosen and how effectively the program is implemented (Swan et al 1992). Result from previous studies have something to recommend for inclusion of Holstein breed, because the impact of percentage of Holstein was dramatic on the traits directly associated with milk production (E.Wall et al 2005). Dairy producer in kermanshah have become increasingly interested in crossbreeding for improving of milk production. The purpose of this study was to estimate the effects of crossbreeding and proportion of Holstein on Iranian native cattle fertility and production.

Materials and Methods. In this experiment four hundred native cows (320±30kg) were used during a cattle breeding program. Treatment groups were 1; native cows, 2; F1 generation and 3; F2 generation. A number of fertility traits were defined including: conception rate, birth weight, milk yield, milk fat percentage, Average number of A.I services, calf mortality, stillbirth and some reproductive abnormality and problems like dystocia and retained placenta. The rectal palpation technique was used for pregnancy detection. The first generation crosses involved native and Holstein mated to Holstein (A.I sires) and backcrosses to Holstein were in the next generation. The F test was used to detect significant differences between means of treatment(native,F1 and F2 dairy cattle)data analyzed using fit model procedure of proc ANOVA (SAS 1997 V.6.12) at the level of $p<0.01$ in completely randomized design, and Duncan's mean test.

Results. Crossbreeding had not a significant effect on conception rate, calf mortality and milk fat percentage (table1) . Services per conception required for native and F1 generation (Average number of A.I services**)** was lower than($P<0.05$) in F2 generation (1.81 and 1.79 respectively vs. 1.88). Crossbreeding had a significant favorable effect on milk production and birth weight ($p<0.01$). Dystocia was more common in native cows ($p<0.01$). The twinning rate and born blind rate were only 1 and 1 case in F1 and F2 generation respectively compared to X in native breed.

Table 1 The effect of crossbreeding on reproduction and production parameters.

Treatments	Traits					
	Milk yield(kg)	Birth weight (kg)	Fat-milk (%)	Conception rate (%)	Calf mortality(%)	Dystocia(%)
Native	6±.80[a]	20± 2[a]	4± .10[a]	55.18±.13[a]	3.43±.7[a]	7.14±.61.[a]
F1	14± .91[b]	30± 2.3[b]	3.8± .15[a]	56.99±.22. [a]	2.42±.81[a]	3.50±.71[b]
F2	15± .93[b]	33± 2.5[b]	3.7± .22[a]	53.55±.26 [a]	2.73±.88[a]	2.99±.82[b]

Values are means ± SEM. Means with different superscripts on the same column are significantly different ($p<0.01$)

Conclusion. It appears that less calving difficulty exists with high percent Holstein genes. Experiment indicated a higher survival rate and adaptation ability when Holstein sire was backcrossed. It seems that inbreeding increase with time and this can be a reason for the estimated favorable effect of low inbreeding on milk yield, calf mortality and stillbirth rate. Producers who responded to this study tended to make heavy use of Holstein AI sires for the F1 and backcross matings. Based on this study it appears that crossbreeding can play a role in improving the profitability and longevity of native cattles.

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The role of zearalenone and β-carotene in heat expression

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Introduction Poor heat detection has been identified as one of the major factors contributing to the current infertility problem, with average heat detection rates on farm of less than 50% in the UK dairy herd. A significant challenge in oestrus detection on farms is the large variation in both the intensity and duration of oestrus behaviour between individual animals, what we have termed, the level of heat expression. As the time and skill available on farms to spend on heat detection declines, the level of heat expression becomes an increasingly important factor in detection rates and thereby cow longevity and herd profitability. However, it has been suggested that, due to a variety of factors, the intensity and duration of oestrus behaviour is reduced in modern dairy cows compared to those observed in cows 20-30 years ago (Mayne 2006). Identifying these factors and finding effective means to control them is therefore a key to success in the modern dairy herd. This contribution will focus on two nutritional factors, specific mycotoxins and β-carotene deficiency, that can influence heat expression in dairy cows but so far have been less well documented in the literature. A novel nutritional approach to enhance heat expression is discussed.

Hidden factors affecting heat expression There are many obvious factors affecting heat expression of cows, e.g. the total number of cows bulling together, poor floor surfaces, loafing area, stress and high milk yields (Kingshay 2006; Mayne 2006). In the UK however, new factors are coming in to play due to major changes in dairy rations.
As herd size increases the trend is towards reduced dependence on grass and increased feeding of wholecrop cereal and maize forages and TMR containing small grain cereals. This combination provides an increased risk for mycotoxin contamination and β-carotene deficiency to occur in dairy rations, both of which can have similar negative effects on heat expression in the dairy cow. β-carotene is abundant in fresh grass but levels decline when grass is ensiled (Immig 2005). Maize and whole crop wheat silages contribute very little β-carotene to the diet. β-carotene deficiency in dairy cows has been associated with poorly recognisable oestrus (Tekpetey *et al*. 1987). Maize and wheat crops are also more susceptible to fusarium mould which can produce mycotoxins, one of which is zearalenone. The structure of zearalenone is very similar to oestrogen and so can have a direct effect on heat expression and other hormonal changes related to fertility (Diekman and Green 1992). Data from analysis of TMR samples taken in the UK will be presented, showing that zearalenone was often present in UK TMR and maize silages analysed in 2006 and 2007.

Novel approach to enhanced heat expression by nutrition Modern dairy cow rations increase the risks of both β-carotene deficiency and zearalenone exposure, two nutritional factors which can have similarly negative effect on heat expression in dairy cows. Effective control measures are therefore required. Adding clays and minerals to dairy cow rations has proved a successful means of binding certain mycotoxins in the rumen e.g. aflatoxins, neutralizing their potentially harmful effects. Research carried out with zeralenone, however, using a great number of clays and minerals, has shown it to be much more difficult to bind. A new, more effective, strategy was developed to deal with zearalenone in the animal (Cast Report 2003). Combining this strategy with dietary supplementation of β-carotene provides a novel dietary means of enhancing heat expression in dairy cows.

Conclusion A higher level of heat expression in dairy cows can help to increase the efficiency of heat detection and thus herd fertility and profitability. Hidden factors affecting heat expression, such as β-carotene deficiency and zearalenone exposure, are best controlled by preventive dietary means in TMR.

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