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Repeatability and heritability of metabolic hormones in the cow

Key external stakeholders:

Dairy and beef farmers, Irish Cattle Breeding Federation (ICBF), nutrition, physiology, genetic and molecular scientists.

Practical implications for stakeholders:

This study showed that IGF-1 has potential as a predictor of cow fertility due to its moderate repeatability and well documented association with a number of reproductive processes. Sampling cows between 2 and 4 weeks postpartum for IGF-1 could help identify cows that are more resilient to the effects of negative energy balance, thereby providing valuable additional information for breeding programmes and assisting selection for improved productive and reproductive potential. We also identified potential causative polymorphisms which, if augmented with functional genomic studies and validated in independent populations of cattle, would yield greater insight into the influence of variants of *IGF-1* and *GH1* on cow performance. While there is considerable variability between animals in the duration of heat and time of ovulation, neither were related to subsequent embryo survival. Furthermore, the study showed that successful pregnancy was possible even when ovulation occurred 31.5 h after AI. This suggests that there would be no detrimental effect of herds adopting once-daily AI, provided that oestrus detection is accurate.

Main results:

This study shows clearly that key metabolic changes that occur in the GH/IGF axis during the early postpartum period are associated with fertility traits. Circulating concentrations of IGF-1, insulin and glucose, indicators of energetic status, have direct and indirect effects on reproductive function. Of all the variables measured, IGF-1 has most potential as a predictor of cow fertility due to its moderate repeatability and well documented association with a number of reproductive processes. Increased circulating concentrations of IGF-1 during the early post partum period had concurrent and latent effects on reproductive events, leading to a shorter interval to first ovulation and conception, improved first service conception rate and overall pregnancy rate.

Two *IGF-1* SNPs, *IGFi1* and *IGFi2* were associated ($P<0.05$) with body condition score at calving while a single *IGF-1* SNP, *IGFi3*, was associated ($P<0.05$) with milk production including milk yield (± 751 kg), fat yield (± 21.3 kg) and protein yield (± 16.5 kg) per lactation. Only one *GH1* SNP, *GH33*, was associated ($P<0.05$) with milk protein yield in the second lactation (allele substitution effect of 9.8 kg). Several *GH1* SNPs were associated ($P<0.05$) with fertility, including *GH32*, *GH35* and *GH38* with calving to third parity (± 22.4 days) (*GH32* and *GH38*), pregnancy rate to first service (± 0.1 %) and overall pregnancy rate (± 0.05 %).

There was no effect of the intervals from the onset of heat to AI or ovulation or from AI to ovulation on embryo survival ($P > 0.10$). There was a tendency ($P = 0.09$) for an inverse relationship between preovulatory follicle size and embryo survival that was unrelated to concentrations of oestradiol or IGF-1 during the periovulatory period ($P > 0.05$). There was evidence ($P = 0.08$) of a positive association between embryo survival and concentrations of progesterone on Day 7, though this relationship was independent ($P < 0.05$) of hormonal and follicular measurements during the periovulatory period. This study shows that heifers could be inseminated up to 31.5 h before ovulation without compromising the probability of embryo survival.

Opportunity / Benefit:

Provides an opportunity to identify animals at a young age that are likely to have subsequently superior reproductive performance. This could be invaluable in selecting cows with greater reproductive efficiency. This is of immediate practical relevance to Irish dairy and beef herds because of the central role of reproductive performance in determining productive and economic efficiency in Irish seasonal calving systems.

Contact Michael G Diskin Email: Michael.diskin@teagasc.ie

Collaborating Institutions:

University College Dublin

Teagasc project team: Dr. Michael G Diskin (PI)
Dr David Kenny,
Dr. Sinead Waters,
Dr. Dermot Morris,
Dr Frank Buckley
Dr Brendan Horan,
Dr Stuart Childs,
Dr Dawn Howard,
Padraic O'Boyle
Ciarán Lynch (WF)

External collaborators: Prof. DA McHugh,
Prof Alex Evans
Dr D. Magee (UCD)

1. Project background:

Reproductive efficiency in both dairy and beef cow herds is a major factor affecting the economic and biological efficiency of milk and beef production, particularly in seasonal calving herds. In dairy cows the decline in conception rate to a single service is the single most important contributor to the decline in overall herd reproductive efficiency. This decline in conception rate has been ongoing for the past three decades, with an average annual decline of 0.5 to 1 percentage points recorded over the past decade; it is currently estimated to cost the Irish cattle industry in excess of €400 million annually. Most discussion about the decline in dairy cow fertility has focused on the possible effects of lactation and or nutrition. Changes in metabolic hormones are dynamic in the post-partum dairy cow and largely reflect the shifting metabolic status of the cow at this time. Blood concentrations of insulin-like growth factor (IGF-I) and insulin decrease immediately after calving and then gradually increase as lactation progresses. These hormones are under metabolic regulation and can influence GnRH secretion by impacting on the neuronal pathways controlling its release. This in turn may alter LH and FSH secretion, the regulators of ovarian follicular dynamics and ovulation. Increased duration of anovulation was associated with decreased concentrations of IGF-I and IGFBP-3 in the follicular fluid of beef cows. There was also evidence that both insulin and IGF-I directly increase the sensitivity of the ovary to gonadotrophins. At the cellular level, physiological concentrations of insulin and IGF-I interact to stimulate oestradiol production by granulosa cells. Irrespective of which pathways insulin and IGF-I operate, there is some evidence that low blood concentrations of both are associated with prolonged postpartum anoestrous intervals in both beef and dairy cows. As well as the roles of IGF-I and insulin in regulating ovarian function and onset of oestrous cycles post-partum, there is emerging evidence of an association between blood concentration of these hormones and conception rate. Both Teagasc and UK studies have recorded a positive association between blood concentration of IGF-I during the early post-partum period and subsequent conception rate. Similarly, in beef heifers, positive association between blood concentrations of IGF-I and embryo survival rate has been reported. The appropriate incorporation of such easily measurable traits as blood insulin and IGF-1 into breeding programmes has the potential to increase the accuracy of selection, provide more accurate breeding values for fertility traits, and at younger ages. This will shorten generation interval or allow more animals to be evaluated, thereby increasing selection intensity. However, further studies are required to clarify the genetic control of IGF-I and determine how best to use systemic insulin and or IGF-I as potential indicator traits to improve reproductive efficiency.

2. Questions addressed by the project:

- What is the relationship between metabolic hormones, metabolites and milk production variables during early lactation and subsequent fertility in lactating dairy cows managed at pasture?
- What are the repeatability values for these hormones and metabolites during this early post-partum period?
- What are the associations of SNPs in bovine growth hormone (GH1), insulin-like growth factor I (IGF-1) genes with direct performance trait measurements of lactation and fertility in Holstein-Friesian cows?
- What are the relationships between periovulatory endocrine events, ovarian activity, and embryo survival after AI in cattle?

3. The experimental studies:

A total of 3 studies were carried out.

Contact Michael G Diskin Email: Michael.diskin@teagasc.ie

Study 1. A total of 371 spring calving multiparous Holstein-Friesian dairy cows, in 7 dairy herds were blood sampled within 8 days of calving and at weekly intervals thereafter, until 4 weeks post calving. Cows were body condition scored (BCS) on a scale of 0 – 5 on Week 1 and Week 4. Cows were bred by AI at a spontaneous oestrus and scanned for pregnancy at 30 – 60 days post AI. Milk production and composition was determined from on farm milk recording. Blood concentrations of total IGF-!, insulin glucose, NEFA, BHB and urea were determined. The repeatability of all metabolic hormones and metabolites across the four-week sampling period was calculated as the ratio of the within group variance to the sum of the within and between group variances. From the on-farm insemination records and detailed pregnancy scans, it was possible to determine the result of each insemination for all cows and this was subsequently confirmed by calving records in the following year. From these data, the following reproductive variables were calculated: postpartum interval to first service (PPSERV1) and days from calving to conception interval (CCI), conception rate to 1st service (PD1) and pregnancy rate (PDFINAL) for all services combined. The relationships between the dependant binary variables and the continuous independent variables IGF1, insulin, glucose NEFA, BHB, urea, BCS and all milk related variables were evaluated using logistic regression.

Study 2. A total of 16 SNPs in both the IGF-1 and GH1 genes were genotyped across 610 cows and association analyses carried out with traits of economic importance including: calving interval, pregnancy rate to first service and 305-day milk production using mixed animal linear models accounting for additive genetic effects (ASREML).

Study 3. A total of 84 beef heifers were oestrus synchronized using a prostaglandin-based regimen. AI was performed between 5 and 21 h after heat onset. Ultrasonic examination of ovarian structures began 12 h after the onset of heat and continued every 6 h until confirmed ovulation. Blood samples were collected for measurement of oestradiol, progesterone, and IGF-1. Pregnancy diagnosis was conducted on Days 30 and 100 after AI. Embryo survival was defined as the presence of an embryo with a detectable heartbeat in a clear amniotic sac at Day 30 post AI.

4. Main results:

Study 1

- Plasma concentrations of IGF-1 in weeks 2, 3, 4 (and the mean of weeks 1 to 4 post calving), and concentrations of glucose (weeks 1, 2, 3 and the mean weeks of 1 to 4 post calving) and urea (week 3 post calving) were positively related to with first service conception rate. Conception rate to first service was also positively related to yields of milk, fat, protein and lactose, as well as energy output during the 3rd month of lactation. Concentrations of IGF-1 were negatively associated with commencement of oestrous cycles. Calving to first service and calving to pregnancy intervals were negatively associated with concentrations of IGF-1 (weeks 1 to 4 and mean of weeks 1 to 4). Conversely, there was a negative association with concentrations of urea (week 3 and 4). Body condition score was negatively association with calving to first service and calving to pregnancy intervals (week 1 and 4).
- IGF-1 during the early post partum period had a moderate repeatability (0.63); repeatability estimates for the other analytes were lower (0.34 to 0.47).
- Systemic concentrations of IGF-1 in early lactation may be a useful metabolic indicator of the subsequent reproductive performance potential of dairy cows.

Study 2

- Two IGF-1 SNPs, IGFi1 and IGFi2 were associated ($P<0.05$) with body condition score at calving while a single IGF-1 SNP, IGFi3, was associated ($P<0.05$) with milk production including milk yield (± 751 kg), fat yield (± 21.3 kg) and protein yield (± 16.5 kg) per lactation.
- One GH1 SNP, GH33, was associated ($P<0.05$) with milk protein yield in the second lactation (allele substitution effect of 9.8 kg).
- Several GH1 SNPs were associated ($P<0.05$) with fertility, including GH32, GH35 and GH38 with calving to third parity (± 22.4 days) (GH32 and GH38 only), pregnancy rate to first service (± 0.1 %) and overall pregnancy rate (± 0.05 %).
- Results of this study demonstrate the direct effects of variants of the somatotrophic axis on milk production and fertility traits in commercial dairy cattle.

Study 3

- There was no effect of the intervals from the onset of heat to AI or ovulation or from AI to ovulation on embryo survival ($P > 0.10$). There was a tendency ($P = 0.09$) for an inverse relationship between preovulatory follicle size and embryo survival that was unrelated to concentrations of oestradiol or IGF-1 during the periovulatory period ($P > 0.05$).
- There was evidence ($P= 0.08$) of a positive association between embryo survival and concentrations of progesterone on Day 7; however, this relationship was independent ($P < 0.05$) of hormonal and

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follicular measurements during the periovulatory period.
- This study shows that heifers could be inseminated up to 31.5 h before ovulation without compromising the probability of embryo survival.

5. Opportunity/Benefit:

The results of this research have significantly extended our understanding of relationships between metabolic hormones, metabolites and milk production variables during early lactation and subsequent fertility in lactating dairy cows. It indicates that IGF-1 measurements during early lactation is moderately repeatable and is a useful predictor of subsequent reproductive performance. The study identified the effects of single nucleotide polymorphisms (SNPs) of the somatotrophic axis on milk production and fertility traits in commercial dairy cattle and these following further examination could be incorporated into breeding programmes. While considerable variability exists between individual animals in the duration of heat and time of ovulation, neither affects subsequent embryo survival. Furthermore, it was possible to establish a pregnancy when ovulation occurred 31.5 h after AI. This suggests that there would be no detrimental effect of herds adopting once-daily AI, provided that oestrus detection is accurate. This is of immediate practical relevance to Irish dairy and beef herds because of the central role of reproductive performance in determining productive and economic efficiency in Irish seasonal calving systems.

6. Dissemination:

National Conferences and seminars
Presented at the Agricultural Research Forum and International conferences such as the World Buiatrics Conference.

Open Day Farmer Discussion Groups
The results of these studies have informed the presentations at Beef Open in Grange and have been incorporated into presentations at Discussion Group and Farmer and industry seminars.

Main Publications:

Mullen, M.P., Lynch, C.O., Waters, S.M., Howard, D.J., O'Boyle P., Kenny, D.A., Buckley, F., Horan, B., Diskin M,G. (2011). Single nucleotide polymorphisms in the growth hormone and insulin-like growth factor-1 genes are associated with milk production, body condition score and fertility traits in dairy cows. *Genetics and Molecular Research*, 10:1819-1830.

Lynch, C.O., Kenny, D.A., Childs, S. and Diskin, M.G. (2010). The relationship between periovulatory endocrine and follicular activity on corpus luteum size, function, and subsequent embryo survival. *Theriogenology*, 73:190-198.

Diskin, M.G. and Morris, D.G. (2008). Embryonic and early foetal losses in cattle and other ruminants. *Reproduction Domestic Animals*, 43 (Suppl. 2): 260-267.

Technical publications:

Diskin, M.G. (2011) Reproductive Management of Dairy Cows. Dairy Encyclopaedia Page 475-484

Diskin, M.G. (2008). Reproductive management of dairy cows: A review (part I). *Irish Veterinary Journal*, 61: 233-239.

Diskin, M.G. (2008). Reproductive management of dairy cows: A review (part II). *Irish Veterinary Journal*, 61: 375-383.

7. Compiled by: Dr Michael G Diskin

Contact Michael G Diskin Email: Michael.diskin@teagasc.ie