Project number: 5517
Funding source: Teagasc

Date: August, 2012
Project dates: Sept 2008 -Aug 2012

Examination of the molecular control of uterine function and fertility in cattle

Key external stakeholders:

Livestock industry, Universities, Veterinarians, AI industry, Department of Agriculture, Food and the Marine, Irish Cattle Breeding Federation (ICBF).

Practical implications for stakeholders:

- A panel of genes and pathways involved in metabolite transport, lipid metabolism and inflammation has been identified which may serve as potential molecular markers for fertility in cattle.
- Genetic variants in the genes *GALNT6, FST, DAP* and *ALB* exhibited significant associations with calving interval.
- Genetic variants associated with positive effects on fertility will be incorporated into the generation of a customized SNP chip for genomic assisted cattle breeding in Ireland. Data will ultimately contribute to the genomic selection programme, accelerating the rate of genetic gain in the Irish cattle population.

Main results:

- A number of genes and pathways affecting uterine function and potentially uterine-mediated conception rate variance were differentially expressed during the mid- and late-luteal phase in cattle of high and low fertility. Genes coding for metabolite transporters, specifically the solute carrier (SLC) family members were found to be differentially expressed between high and low fertility animals.
- The gene, *GALNT6* was decreased 7-fold in high compared with low fertility heifers. Expression of this gene is implicated in the synthesis of oncofetal fibronectin which is a protein found in plasma and cervicovaginal secretions; increased concentrations of which has been found in abnormal pregnancy states. In addition, *GALNT6* variants exhibited a significant association with calving interval.
- Variants of the genes *FST*, *DAP* and *ALB*, were also strongly associated with calving interval.

Opportunity / Benefit:

Key genes have been identified which may serve as molecular markers for cow fertility and following validation, such markers could be incorporated into future cattle breeding programmes to improve the reliability of predictions and increase the rate of genetic gain for fertility related traits.

Collaborating Institutions:

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1. Project background:

Cow reproductive efficiency is important to both the beef and dairy cattle enterprises. When there is sub-optimal reproductive performance it can be a significant cost to these multi-million euro industries. The aim of this project was to investigate a critical component of the reproductive process, with the ultimate aim of increasing our understanding of the genomic control of uterine function and overall cow fertility.

The bovine endometrium has received much attention because of its critical influence on embryo and foetal growth and survival. In particular, this outermost layer of the uterus maintains the conceptus through it's nutrient rich histotroph secretions and provides a structural point for embryonic implantation. Given the importance of this tissue in reproduction, it is important to gain a greater understanding of uterine function. The greatest increment of cow reproductive wastage occurs in the form of embryo mortality, particularly in early pregnancy prior to initiation of implantation. The extent and timing of this has been closely examined in both beef and dairy herds and it can be concluded that the majority of embryo loss occurs within 14-16 days following insemination. Specifically, it has been reported that most embryonic mortality occurs between day 8 and 16 after AI and is thus it is termed "early embryo loss". Considering the nature of its timing, examination of molecular events regulating the initiation and culmination of this early embryo loss, is a priority in reproductive biology and as such days 7 and 14 of the oestrous cycle were targeted in this study. Taking these factors into account, the aim of this project was to elucidate the key genes, pathways and genetic variants potentially regulating and influencing fertility traits like early embryo survival, conception rate, calving interval and general uterine endometrial function in cattle.

2. Questions addressed by the project:

Do endometrial gene expression profiles differ between heifers ranked as either high or low for fertility during the mid-luteal phase of the oestrous cycle (day 7)?

Do endometrial gene expression profiles differ between heifers ranked either high or low for fertility during the late-luteal (day 14) phases of the oestrous cycle and in turn how do these compare this with the expression profiles from the mid luteal phase?

Do variants of the genes, identified as being differentially expressed between heifers of either high or low fertility occur and are these different in bulls categorised as high and low genetic merit for calving interval, and how are these genetic variants associated with reproductive and other performance traits?

3. The experimental studies:

Reproductively normal crossbred beef heifers (n=120) were inseminated and pregnancy diagnosis was carried out 28 days later, after which animals were programmed to return to oestrus. Animals were re-inseminated followed by pregnancy diagnosis on a further four occasions. On the basis of pregnancy rate to 4 successive inseminations, animals were divided into two groups: i) those that established pregnancy on all 4 occasions (high fertility group) and ii) animals achieving pregnancy on only one occasion (low fertility group). Oestrous cycles were then synchronised following administration of a prostaglandin F2a analogue (Estrumate®). Animals from both high and low groups (n=12) were slaughtered on day 7 of their oestrous cycle and uterine endometrial tissue (ipsilateral to corpus luteum) was harvested and stored at -80ºC in RNAlater solution. This was repeated with animals slaughtered and tissue collected on day 14 of the oestrous cycle. RNA was extracted, purified and DNAse treated. RNA quantity and quality was determined using spectrophometry and the 2100 Agilent. RNA samples were biotinylated and hybridised to the Affymetrix 23,000 bovine master gene chip according to manufacturers' protocol. The microarrays were scanned and data normalised using the array analyser module of SPLUS and R. Statistical analysis and controlling for false discovery was carried out using the PUMA method in Bioconductor, R. Furthermore Ingenuity Pathway Analysis (IPA) was used to associate changes in gene expression with key biochemical pathways involved in global biological function in the bovine uterus.

A DNAseq approach incorporating DNA pooling, target enrichment and high throughout sequencing was applied to identify polymorphisms and estimate allele frequencies in 10 candidate genes found to be differentially expressed in microarray studies. DNA samples from 150 Holstein-Friesian bulls divided into 2 groups (n=75) divergent (high and low) for genetic merit for calving interval were pooled using equimolar quantities from each animal to construct 2 Illumina® sequence libraries. A custom Agilent Technologies SureSelect™ Target Enrichment System was used to selectively capture and enrich coding, intronic and regulatory sequences of the targeted genes. These enriched libraries were then sequenced using an Illumina® Genome Analyzer II. Genetic variants with significant (≥2-fold; P<0.01) allele frequency differentials between the low and high calving interval (LCIV and HCIV, respectively) groups were genotyped in 848 dairy

bulls and association studies conducted to examine the effect of these genetic variants on fertility and other performance traits in cattle.

4. Main results:

Microarray analysis detected 419 differentially expressed genes (DEG) between high and low fertility animals on day 7 of the estrous cycle. The main gene pathways affected were, cellular growth and proliferation, angiogenesis, lipid metabolism, cellular and tissue morphology and development, inflammation and metabolic exchange. DEG included, *FST, SLC45A2, MMP19, FADS1* and *GALNT6*. 430 DEG were detected between high and low fertility animals on day 14. Pathway analysis revealed enrichment for many biological pathways including lipid metabolism, molecular transport, immune response, cell morphology and development, cell growth and proliferation and reproductive function. Important DEG included *ALB, BMPR2, CCL28, COL4A3/4, FADS1, ITGA6, LDLR, PCCB, PLCB3*, *PPARG, PTGS2* and *SLC27A4*. The present study highlights some of the pathways and mechanisms underpinning late-luteal bovine endometrial physiology and endometrial related conception rate variance.

DNA-Seq and subsequent association analysis of variants contained in *GALNT6* revealed several significant associations with a variety of reproductive variables. In particular, significant associations were observed between 4 SNPs in *GALNT6* and calving difficulty and gestation length, while positive associations were observed with calf difficulty and mortality. Furthermore, all of these variants resulted in non-synonymous substitutions ultimately resulting in a change in protein sequence and therefore potentially function. Another variant in *GALNT6* exhibited a significant association with increased calving interval and decreased cow survival. This specific variant also results in a non-synonymous substitution, with a 2-fold allele frequency differential between original high and low CIV animals.

Two variants in the *FST* gene, and one in each of *DAP* and *ALB* were strongly associated with shorter calving intervals. Also, DNA sequencing analysis revealed a 2.29 and 2.28 allele frequency differential for the two *FST* SNPs between HCIV and LCIV animals, respectively. The physiological role of *FST* has been extensively investigated in numerous species. *FST* is imperative in daily cellular homeostasis in early reproductive organ development and subsequent fertility. Furthermore high vs low fertility heifers exhibited differential endometrial expression of *FST*. Furthermore, *FST* was found to be expressed in endometrium, during implantation and when treated with steroid hormones oestradiol and (or) progesterone, reinforcing the concept that this gene may play a critical role in bovine endometrial function and fertility.

5. Opportunity/Benefit:

Key genes have been identified which may serve as molecular markers for fertility. Following validation, such markers could be incorporated into future cattle breeding programmes to improve the reliability of predictions and increase the rate of genetic gain for fertility related traits.

6. Dissemination:

Killeen, A.P. (2012) 'Genomic control of uterine function and fertility in cattle.' Thesis, UCD.

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Killeen, A.P., Mullen, M.P., McCabe, M.S., Kenny, D.A., Magee, D.A., Creevey, C.J., MacHugh, D.E. and Waters, S.M. (2011) 'Allele frequency differentials of regulatory polymorphisms across 10 candidate fertility genes in dairy cattle divergent for calving interval.' *Proceedings from the 4th International Symposium on Animal Functional Genomics*, Dublin, Ireland. 10th October.

Killeen, A.P, Morris, D.G, Kenny, D.A, Diskin, M.G and Waters, S.M. (2010) 'Uterine endometrial gene expression in heifers of high or low fertility on day 14 of the oestrous cycle.' *Proceedings from the BSAS and Agricultural Research Forum,* Belfast, 12th March, p161.

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