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Mutations in genes involved in oestrous cycle associated expression of oestrus

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ABSTRACT

Detection of oestrus is a key determinant of profitability of dairy herds, but oestrus is increasingly difficult to observe in the modern dairy cow, with shorter duration and less intense oestrus. Concurrent with the unfavourable correlation between milk yield and fertility, oestrous detection rates have decreased to less than 50%. A number of mutations have been identified in genes associated with fertility and production traits, but, to date, no single nucleotide polymorphism (SNP) has been associated with oestrous expression. Therefore, the objective of this study was to investigate SNPs, linked to fertility, for the association with oestrous expression. Blood was collected from 205 Holstein Friesian dairy cows and genotyped at 41 loci of 18 genes chosen for their roles in the oestrous cycle and milk production. SNPs were then examined for correlations with increase in activity at oestrus, recorded via activity monitors, using generalised linear models. Physical activity increased at oestrus between two and four fold. Larger increases were associated with mutant alleles in oestrogen receptor-α and gonadotrophin releasing hormone receptor genes ($P < 0.05$) and in the *STAT5A* gene ($P < 0.05$). Smaller increases were associated with mutant alleles of the activin receptor type IIB and prolactin receptor genes ($P < 0.10$). In conclusion, alleles in these five genes provide the opportunity for selection of animals displaying greater oestrous activity which could aid reversal of the decrease in oestrous detection and thereby contribute to sustainability of the dairy industry worldwide.

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1. Introduction

Oestrous detection is key to profitability of dairy herds (Pecsok et al., 1994), but oestrous detection is becoming increasingly difficult due to decreased expression in the modern dairy cow. Cows are reported to display shorter duration and less intense oestrus (Dransfield et al., 1998), where fewer cows stand to be mounted (Dobson et al., 2008). The decrease in fertility associated with increases in milk yield (Royal et al., 2000; Pryce et al., 2004) is thought to have a strong genetic basis (Veerkamp and Beerda, 2007) and it is apparent that a genetic approach may be employed to solve the problem. It appears that poor expression of oestrus is 50% of the problem contributing to poor dairy cow fertility, as only 50% of cows are detected in oestrus (Van Eerdenburg et al., 2002). Sequencing of the bovine genome in 2003 (http://www.hgsc.bcm.tmc.edu/projects/bovine) has provided a wealth of information at a molecular level and it is possible that genomic approaches might provide a novel solution to the problem of poor expression of oestrus.

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There are many effects of single nucleotide polymorphisms (SNPs) and so relationships can be determined between SNPs and a functional trait (Ibeagha-Awemu et al., 2008). A number of SNPs have been identified and reported for their association with reproductive and production traits (Table 1); however, as yet there is no report of effects of SNPs on oestrous expression. Based on a literature review and in-house genotyping, genes were chosen for analysis in the present study on the basis of the association with (i) hypothalamic/ovarian/uterine function, (ii) roles in central nervous pathways controlling oestrous behaviour, and (iii) associations with production traits such as milk yield, energy balance and feed intake and metabolic influences which can all impact upon fertility and oestrous expression. Therefore SNPs on genes that had been identified previously (Table 1) were investigated for the effects on physical activity at oestrus.

Selecting for increased activity at oestrus may lead to an increase in overall oestrous expression and thereby aid a more rapid reversal of decreasing oestrous detection rates. Due to the low heritability of reproductive traits ($h^2 < 0.05$; Berglund, 2008), genomic selection provides a method of rapid and cumulative genetic gain with the possibility of doubling the rate of genetic gain (Hayes et al., 2009). Furthermore the accuracy of SNP selection has been reported to be increased between 10% and 30% for traits with low heritability (Muir, 2007).

By improving oestrous expression, more cows would be detected in oestrus and thus oestrous detection rates would be improved. Identifying SNPs for strong oestrous expression would allow selection of bulls for breeding that would produce cows that display greater oestrous behaviour. Therefore, the objective of the present research was to identify DNA polymorphisms that could provide a means of identifying cows that display oestrous expression more strongly.

2. Materials and methods

2.1. Animals

Animals used in the present study were 205 Holstein Friesian dairy cows housed at the Nottingham University Dairy Centre (Sutton Bonington, Leicestershire, UK; average annual milk yield 10,000 L/cow). The lactating cows were kept indoors in groups of approximately 40. Housing consisted of a purpose built barn with four pens, which was well ventilated, with rubber matting, cubicles and sawdust for comfort whilst lying. Cows were milked by robotic automatic milking stations (Lely Astronaut A3 AMS units) voluntarily; visiting between two and six times per day. All cows were fed the same silage-based diet, with concentrates fed in the robot at milking.

2.2. Phenotypic data

Physical activity of each cow was monitored continuously by a Lely Qwes-HR Activity Tag mounted on a neck collar. The tag contains a three-dimensional accelerometer which responds to cow movement and movement intensity. Accelerometer signal is converted to an activity index by an internal algorithm, which is patented and undisclosed by the manufacturer. Activity data, therefore, are expressed as 'activity units', but cannot be translated into any specific activity measure such as number of steps or shakes of the head. Activity data were downloaded at milking from the tag, which was read on entry to the robotic milking station.

Activity data were recorded for each cow as 12 readings daily of mean activity units per 2 h interval. Activity data were analysed by plotting activity against date and time resulting in 12 activity readings daily with peaks denoting oestrus. An increase in activity at oestrus was defined as three consecutive 2-h periods of increased activity compared with the baseline before onset of increased physical activity. The threshold for physical activity increase to be considered a possible oestrous event was 30% above baseline.

Peaks in activity were confirmed as oestrus events by visual detection (observations in the morning; 06:00 h and evening; 20:00 h during daily routine activities). Only peaks after 25 days postpartum were classified as oestrus. Any increase in activity within 10 days postpartum was discounted from analysis as these may be due to management practices or measurement errors and hence not a true representation of oestrous cyclical activity.

Oestrus, insemination and pregnancy data were known for all animals. Oestrous data collected over 2 years were collated from 205 cows, including 930 individual oestrous events across different lactations and stages of lactation. Measurements of oestrous expression were calculated as maximum activity at oestrus.

2.3. Blood sampling, DNA extraction and genotyping

Whole blood samples were collected from the coccygeal vein of each cow into lithium heparin coated tubes, under ethically approved Home Office Licence regulations. Blood samples were then genotyped commercially by KBiosciences Ltd (Herts, UK), using primer extension. Based on a literature review and in-house genotyping in other studies, genes were selected for analysis on the basis of involvement in reproductive processes and are detailed in Table 1. DNA was genotyped at 41 loci, in 18 genes for inclusion in the analysis.

2.4. Statistical analysis

Statistical analysis was conducted using Genstat 14th edition (VSN International Ltd, Hemel Hempstead, UK). Activity data were analysed as generalised linear mixed models (GLMM) using the residual maximum likelihood (REML) procedure, with Poisson distribution and logarithmic link function. The model fitted fixed effects for SNP (wildtype homozygote, 0; heterozygote, 1; mutant homozygote, 2), parity (classified according to lactation number as 1, 2 and $\geq$3) and oestrous season (classified as January–March, 1; April–June, 2; July–September, 3; October–December, 4). Stage of lactation (days in milk) and oestrous number within a lactation were not significant fixed effects, so were omitted from the final model. For the random effects of the model, individual cows

Table 1
SNPs on genes investigated for their associations with an increase in physical activity at oestrus in a herd of 205 Holstein Friesian dairy cows.

Gene name	SNP variant	SNP position	Effects on traits; fertility and production	Reference
Activin receptor type IIB	ACT_IIB_45	Highly polymorphic within intron	Association with reproduction	Flavin et al. (1996)
	ACT_IIB_46			
	ACT_IIB_503			
	ACT_IIB_86_END			
	ACT_IIB_95			
Oestrogen receptor-α	bERA_prom_SNP173	Promoter region, position 173	Oestrogens play a main role in reproduction	Szreder and Zwierzchowski (2004, 2007)
	ESR1 ex1 A503C	Exon 1 A503C		
	ESR1	Exon 8		
Oestrogen receptor-β	bERB_ex4	Exon 4		
	bERB_ex7	Exon 7		
Gonadotrophin releasing hormone receptor	bGNRHE_ex1_SNP_340	Exon 1, position 340	Associations with fertility	Derecka et al. (2009)
	bGNRHR_ex1_SNP_286	Exon 1, position 286		
	bGNRHR_ex1_SNP_421	Exon 1, position 421		
	bGNRHR_ex1_SNP_490	Exon 1, position 490		
	bGNRHR_prom_SNP_1189	Promoter region, position 1189		
	bGNRHR_prom_SNP_966	Promoter region, position 966		
Luteinizing hormone-β	bLHB SNP1588	SNP1588		
Fatty acid synthase	FASN 16009a/g	BTA19, 16009A → G in exon 34	Milk fat content in Holstein Friesians	Roy et al. (2006)
	FASN 763g/c	BTA19, 763G → C in exon 1		
	FASN 17924a/g Thr/Ala	BTA19, g.17924A > to G (Thr → Ala)	Fatty acid composition of milk fat	Morris et al. (2007)
	FASN 18663t/c	BTA19, g.18663T > C	Fatty acid compositions	Zhang et al. (2008)
Follicle stimulating hormone receptor	FSHR_L502L	Leu502Leu	Involvement of genes in the oestrous cycle	Kamila Derecka (2010, personal communication)
	FSHR_N669N	Asn669Asn		
	FSHR_S596S	Ser596Ser		
	FSHR_T658S	Thr658Ser		
	FSHR_T685T	Thr685Thr		
Growth hormone receptor	GHR Phe279Tyr	Phe279Tyr in trans membrane domain	Effect on yield and protein and fat percentage and protein and fat yields Affects feed intake, feed conversion and body energy	Blott et al. (2003), Banos et al. (2008)
	GHRA257G ex10	Exon 10 A857G	Associated with milk fat and protein yields	Kaminski et al. (2006)
Leptin promoter	Leptin_promoter −963	C963T	Milk yield, feed and dry matter intake Association with fertility, energy balance and protein yield	Liefers et al. (2005), Banos et al. (2008)
	Leptin_promoter_1 −1457	A1457G		
Luteinising hormone receptor	LHR_L490L	Exon 11, Leu490Leu	Associations with fertility and production; affecting calving interval, days to first service and production index	Hastings et al. (2006)
	LHR_Q527H	Exon 11, Gln527His		
	LHR_W467C	Exon 11, Trp467Cys		
Neuropeptide Y	npy_ex1	Exon 1	Associations with daily gain, body weight and feed conversion ratio	Sherman et al. (2008a), Bahar and Sweeney (2008)
Neuropeptide Y receptor Y2	NPYRY2			

Table 1 (*Continued*)

Gene name	SNP variant	SNP position	Effects on traits; fertility and production	Reference
Peroxysome proliferator-activated receptor-γ coactivator-1α	PPARGC1A C1892t/c intron9	c.1892+T > C in intron 9	Association with milk fat yield	Weikard et al. (2005)
Prolactin	PRL 89398g/a R	G8398A	Milk yield and fat percentage in 1st lactation	Brym et al. (2005)
Prolactin receptor	PRLR Ser18Asn	Ser18Asn in signal peptide	Associated with milk protein and fat yields	Viitala et al. (2006)
Ribosomal protein S6 kinase	rs29019569CT	BTA2, C > T, base pair position 316 880	Effects on feed efficiency	Sherman et al. (2008b)
Signal transducer and activator of transcription 1	STAT1 c3141t	C3141T in 3′ UTR	Allele C associated with increases in milk fat and protein percentages	Cobanoglu et al. (2006)
Signal transducer and activator of transcription 5A	STAT5A g12195c	G12195C in exon 8	Associated with decreases in milk protein and fat percentage Associated with embryonic survival	Khatib et al. (2008), Khatib et al. (2009)

represented subjects to allow for multiple oestrous events per cow. The significance of fixed effects was assessed by Wald tests. The resulting model was:

$$Y_{ijkl} = \mu + S_i + P_j + O_k + C_l + e_{ijkl}$$

where Y_{ijkl} is maximum activity at oestrus, the fixed part of the model consists of; μ the overall mean, S_i the effect of SNP, P_j the effect of parity, O_k the effect of oestrous season, C_l the random effect of Cow, and ε_{ijkl} the residual error.

To investigate possible interactive effects, all combinations of SNPs were added to the single-SNP model, and none was significant.

3. Results

Mean baseline activity before oestrous events was 35 (s.d. 8.3) activity units and ranged from 15 to 72 units. Mean maximum physical activity at oestrus was 68 (s.d. 19.3) activity units and ranged from 26 to 150 units. Mean increase in physical activity at oestrus as a percentage of baseline activity was 87% (s.d. 30%) and ranged from 31 to 200%. The correlation between percentage increase from baseline and maximum activity at oestrus was $r = 0.57$ ($P < 0.001$).

Results are reported for associations between SNPs at 41 loci on 18 genes and maximum physical activity at oestrus. Effects of particular SNPs on physical activity at oestrus where $P < 0.10$ are presented in Table 2 as mean activity at oestrus predicted by the statistical model for each genotype. Only two genes were found to be associated ($P < 0.05$) with activity at oestrus; STAT5A g12195c on the signal transducer and activator of transcription 5A (*STAT5A*) gene and ACT_IIB_95 on the activin receptor type II B (*ACVR2B*) gene (Table 2). Physical activity at oestrus was greater in cows with the mutant genotype (GG) compared to the wildtype genotype (CC) for the *STAT5A* gene ($P = 0.028$). Physical activity at oestrus was less in cows with the mutant genotype (GG) in the *ACVR2B* gene, compared to the wildtype genotype (AA; $P = 0.048$). Three other SNPs on the *ACVR2B* gene tended to be associated ($P < 0.10$) with physical activity at oestrus (Table 2).

SNPs on three other genes also tended towards significance ($P < 0.10$); the mutant genotype of a SNP on the oestrogen receptor-α (*ESR1)* gene and four SNPs on the gonadotrophin releasing hormone receptor (*GNRHR*) tended to be associated with greater activity at oestrus, whereas the mutant genotype of a SNP on the prolactin receptor (*PRLR*) gene tended to be associated with reduced activity at oestrus. No other SNP investigated (Table 1) was significantly associated with activity at oestrus, and no SNP was associated with baseline activity.

4. Discussion

The objective of the present study was to identify SNPs associated with amount of oestrous expression, as indicated by physical activity at oestrus. Physical activity increases at the time of oestrus (Farris, 1954), with activity increasing from two- to four-fold (Kiddy, 1977). Two SNPs on genes *STAT5A* and *ACVR2B* were found to be related to physical activity at oestrus ($P < 0.05$), but several other genes; *ESR1*, *GNRHR* and *PRLR* tended to be associated with physical activity at oestrus ($P < 0.10$). None of other genes investigated (Table 1) had a significant association with oestrous activity.

Polymorphisms in the *STAT5A* gene have been reported to affect fertility by affecting fertilisation rate and embryo survival (Khatib et al., 2009). STAT proteins are involved in cytokine signalling pathways converting signals in the cytoplasm and acting as transcription factors in the nucleus, to regulate gene transcription (Kisseleva et al., 2002). *STAT5A* is activated by more than 35 polypeptide ligands and the resulting gene transcription has involvement in a broad range of physiological responses (Darnell, 1997), such as mediating peptide hormones and cytokines (Selvaggi et al., 2009). The association of *STAT5A* with hormones of the oestrous cycle that influence oestrus could explain the significant effect found in this study. The *STAT5A* gene has been associated with milk composition; the G

Table 2
Associations between SNP and physical activity at oestrus in genes related to the oestrous cycle of Holstein Friesian dairy cows and mean prediction of activity index for each genotype.

Gene	SNP	*P* value	Genotype	Cows, *n*[a]	Mean prediction of physical activity[b]
Activin receptor type 2B (*ACVR2B*)	ACT_IIB_45	0.057	TT	60	68
			TC	97	66
			CC	30	63
			S.E.		1.02
	ACT_IIB_46	0.077	GG	65	68
			GT	93	66
			TT	30	64
			S.E.		1.02
	ACT_IIB_86_END	0.064	AA	65	68
			AG	92	66
			GG	30	64
			S.E.		1.02
	ACT_IIB_95	0.039	AA	60	68
			AG	96	66
			GG	31	63
			S.E.		1.02
Signal transducer & activator of transcription 5A (*STAT5A*)	STAT5A g12195c	0.025	CC	61	68
			CG	99	70
			GG	27	73
			S.E.		1.03
Oestrogen receptor-α (*ESR1*)	bERA_prom_SNP173	0.056	GG	172	68
			GA	16	74
			AA	0	–
			S.E.		1.06
Gonadotrophin releasing hormone receptor (*GNRHR*)	bGNRHE_ex1_SNP_340	0.072	CC	66	69
			CT	89	71
			TT	31	73
			S.E.		1.02
	bGNRHR_ex1_SNP_286	0.079	GG	154	68
			GA	32	72
			AA	1	75
			S.E.		1.04
	bGNRHR_ex1_SNP_490	0.091	CC	66	69
			CT	88	70
			TT	32	72
			S.E.		1.02
	bGHRHR_prom_SNP_1189	0.095	TT	66	69
			TC	89	70
			CC	32	72
			S.E.		1.02
Prolactin receptor (*PRLR*)	PRLR_Ser18Asn	0.053	AA	142	68
			AG	0	–
			GG	39	63
			S.E.		1.02

[a] *n* = number of cows of each genotype in analysis. 205 cows were blood sampled although due to availability of genotyping at each locus the number of cows in each analysis varies.
[b] Physical activity is maximum at oestrus of activity index values recorded by neck-mounted HR Tags containing 3-D accelerometers. Data are means predicted by the statistical model for each genotype, adjusted for effects of parity and season and rounded to the nearest whole number. Standard error (S.E.) is mean prediction error of the model.

allele was linked to low fat and protein percentages in milk (Khatib et al., 2008). Phenotypically, lesser milk protein concentrations are associated with negative energy balance and thus poor fertility, whereas the G allele in the current study was associated with improved oestrous expression.

The other significant SNP found in this study was ACT_IIB_95 on the *ACVR2B* gene. The *ACVR2B* gene has a role in ovarian folliculogenesis and is present on theca cells, granulosa cells and oocytes (Knight and Glister, 2003). Inhibin and activin are the two main ligands for the activin receptor, and have been demonstrated as intra-follicular regulators in ruminants controlling folliculogenesis and steroidogenesis (Hutchinson et al., 1987; Shukovski et al., 1991). Therefore this gene could influence production of oestradiol which stimulates oestrous expression. No other reproduction-related gene was associated with oestrous expression.

The oestrogen receptor (ESR) showed a strong tendency to influence activity at oestrus ($P = 0.056$), which may be confirmed by further investigation into this gene. The ESR is a likely candidate to influence oestrus directly through its presence in the ovary and by interacting with its specific ligand, oestradiol, to influence oestradiol concentrations and the effects of oestradiol (Schams and Berisha, 2002). The importance of the ESR for its role in reproduction is also recognised as this receptor has been targeted for mutations beneficial to farm animal reproduction (Szreder and Zwierzchowski, 2007). Likewise, SNPs on the *GNRHR* are likely candidates to influence the oestrous cycle and thus oestrous expression, which could explain the weak associations ($P = 0.07–0.09$) found in the current study. Gonadotrophin releasing hormone (GnRH) is the major ligand for the *GNRHR* and is a major controller of reproductive processes by regulating gametogenesis and steroidogenesis (Rispoli and Nett, 2005), via the gonadotrophins luteinising hormone and follicle stimulating hormone.

Another relevant SNP is PRLR_Ser18Asn on the prolactin receptor (*PRLR*) gene. SNPs of the *PRLR* have been associated with fertility and embryo survival rate (Khatib et al., 2009). Other reproductive links with the *PRLR* are the influence on prolificacy in sheep (Chu et al., 2007) and effects on ovulation rate in the pig (Van Rens et al., 2003), which demonstrate that the *PRLR* is a possible candidate for influencing oestrous expression, although it did not quite reach significance ($P = 0.053$) in the current study. Reports have suggested that with lesser concentrations of prolactin or the PRLR, oestrous expression may be enhanced (Kommadath et al., 2010), as prolactin has been reported to decrease sexual arousal by inhibiting dopaminergic activity which is excitatory (Bancroft, 2005). Polymorphisms in the *PRLR* have also been related to milk yield. Genotypes AA and AG have been associated with greater milk yield than the GG genotype (Viitala et al., 2006), whereas in the current study the AA genotype was associated with greater expression of oestrus, which is inconsistent with the theory that increased milk yield is associated with decreased fertility. However, the genotypes associated with increased yield were reported for Finnish Ayrshire cattle (Viitala et al., 2006) and were not found in Holsteins (Blott et al., 2003).

Interestingly, none of the genes associated with feed efficiency, increased body weight, increased milk fat and milk protein, conditions most associated with cows that will have greater fertility, was associated with physical activity at oestrus, and only prolactin receptor showed a tendency ($P = 0.053$). Likewise, none of the genes linked to reproduction was associated except for ACT_IIB_95 ($P = 0.039$). The STAT5A mutant was associated ($P = 0.025$); however, that gene is associated with decreased milk fat and milk protein, which is counter-intuitive. Several factors are relevant to this lack of association between SNPs and oestrous expression, but there are three primary considerations. Firstly, although links have been found between physiological mechanisms controlling metabolism and fertility (Garnsworthy et al., 2008), such mechanisms have not been investigated in relation to oestrous expression. It is possible that the link between metabolism and oestrous expression is not as great as that between metabolism and other aspects of fertility. Secondly, it is important to distinguish between phenotypic and genotypic associations. The low heritability of reproductive and productive efficiency traits in dairy cows emphasises the overwhelming contribution of environmental factors in determining fertility and performance. With regard to oestrous expression, Dobson et al. (2008) argue that it is the inability to meet the demands of the high-yielding cow that leads to increased disease incidence, reduced fertility and poor expression of oestrus. This leads to the third consideration, which is the population studied. The number of cows studied was limited, activity at oestrus could only be measured in cows exhibiting oestrus, and all cows were in one well-managed herd, which limits both genetic and phenotypic diversity of the population. Furthermore, fertility studies involving cows used in the current study (e.g. Garnsworthy et al., 2009) have demonstrated that reproduction can be altered independently of milk yield, energy balance and feed efficiency.

Further work is needed to complement and extend the results of the current study. With a larger population, including cows on a range of farms, confirmation of the involvement of SNPs tending towards significance may be realised.

5. Conclusions

The current study identified SNPs on two genes, *ACVR2B* and *STAT5A*, which were associated with activity at oestrus ($P < 0.05$). Other genes *ESR1*, *GNRHR* and *PRLR* may also be associated with the increase in activity at oestrus ($P < 0.10$). Alleles have been identified as those that could be beneficial for oestrous behaviour and provide an opportunity for selection of cows displaying stronger oestrous activity. Genomic selection for these SNPs should result in improved oestrous detection rate and thus improved conception rate, thereby contributing to reversal of the decline in dairy cow fertility.

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