Genome-wide association study of fertility traits in dairy cows

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Implications The markers and genes associated with fertility in dairy cows were identified using SNP chip analysis and a genome wide association study (GWAS) , and will be applied in selection and breeding using marker-assisted selection (MAS) to improve the reproduction of dairy cows.

Introduction The fertility of the dairy cow has declined worldwide in the past decades. Infertility in turn is the major factor causing culling and resulting in reduced longevity. This short herd life decreases the profitability of milk production, resulting in huge economic losses. The development SNP array technology is useful to identify associated markers and genes for the genetic improvement of functional traits in dairy cows. The aim of this study was to perform a GWAS on fertility traits in 495 dairy cows.

Material and methods A total of 495 Holstein cows were recruited, including 263 Chinese Holsteins from one Chinese farm and 232 Holstein-Friesians from 19 UK dairy farms. The following fertility traits were recorded for each cow: (1) days to first service after 1st and 2nd calving (DFS1 and DFS2); (2) days from 1st and 2nd calving to conception (DC1 and DC2); (3) calving interval in days from 1st to 2nd and 2nd to 3rd calving (CI1 and CI2). Genomic DNA was extracted from whole blood using Qiagen reagents according to the manufacturer's instructions. The SNPs were genotyped using the Illumina BovineSNP50 BeadChip in the Department of Pathology, University of Cambridge. Quality control was performed for both samples and SNPs. After quality control, there were 482 individuals and 36,025 SNPs for association analysis. Association was analyzed by a mixed animal model in PLINK 1.07 and ASReml 2.0. This was performed in four steps. (i) The data were analyzed by mixed model and environmental residuals were estimated, excluding the SNP genotype effect at this stage. (ii) These residuals were used as the dependent trait in a simple linear regression for each SNP. All the SNPs were sorted by the original P values. (iii) The top 50 most significant SNPs (SigSNP) for each trait were tested individually in the mixed model and significance was established at $P<0.001$. (iv) Finally, the 4 SNPs adjacent to each SigSNP were tested in the mixed model one by one. If ≥3 SNPs in a group were all significant ($P<0.05$) the SigSNP was selected as a candidate SNP.

Results The analysis identified 24 SNPs which were associated with fertility traits, of which 10 were located in genes: 7 SNPs had significant effect on days to first service (DFS1 and DFS2), 12 SNPs on days to conception (DC1 and DC2) and 5 SNPs on calving interval (CI1 and CI2) (Table 1). Stromal interaction molecule 1 (*STIM1*), a Ca^{2+} depletion sensor, and potassium voltage-gated channel (*KCNQ1*) are both imprinted genes. P450 oxidoreductase (*POR*) knockout is lethal in mice and in humans mutations are associated with abnormal steroidogenesis and infertility. Three nearby genes are involved in immune function (*CD83, CD46, CD96*), while *PLA2G4A* is important for eicosanoid synthesis.

Table 1 Summary of the selected SNPs for each fertility trait of dairy cows

Traits	n	Genes located in	Genes nearby (<500kb)	Traits	n	Genes located in	Genes nearby (<500kb)
DFS1	6	*KCNQ1*	*MAPKAPK2, CD83*	DFS2	1	*AAGAB*	
DC1	6	*MMP20, SBF2, RRM1, STIM1*	*LOC789648, LOC518224(=CD46)*	DC2	6	*RAD54L*	*CD96, STMN2, PLA2G4A*
CI1	2	*GKAP1, N4BP1*		CI2	3	*POR*	*LOC788183, LOC616944*

Conclusion We identified 24 SNPs from the genome-wide study which significantly associated to fertility traits of dairy cows. These markers will be useful for future genetic breeding of dairy cows for better fertility.

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Importance of reproductive traits on the moment of semen acquisition for dairy cattle by public agencies in Western Santa Catarina State - Brazil

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Implications The announcements to buy dairy cattle semen by public agencies of Western Santa Catarina State in Brazil do not consider reproductive traits.

Introduction The dairy production has been occupying a great and significant space in the Brazilian economy, increase in the national production, prominently in Santa Catarina state. In some cities of Santa Catarina State programs funded by the government are the most effective way found by them to improve the genetics of the herds. These programs consists in aquisition of the semen by the government and provide that to the farmers without costs or lower costs. Considering the importance of dairy activity to the regional economy and the impact of animal breeding programs proposed by public agencies through Artificial Insemination (AI), the criteria used for purchase the semen must be monitored. Although the reproductive traits in dairy cattle suffer great environmental influence and consequently low heritability, it is highly important to choose appropriate breeding schemes to improve this traits. The reproductive performance of a herd is one of the most important components in the economic performance of a dairy farm (Leite et al., 2001). The aim of the present study was to verify the importance of reproductive traits in the criteria adopted in the purchase of dairy cattle semen in Western of Santa Catarina State – Brazil.

Material and methods Were obtained the announcements of the top five producing municipalities namely, Chapecó, Xanxerê and Concórdia microregions. Announcements (23) are available on public agencies websites. Was conducted a descriptive analysis of these annoucements focusing in the purchase of bovine semen. This analysis was intended to check the criteria adopted for the acquisition, as Predicted Transmitting Ability (PTA) data, and also to check the announcements which considered any reproductive trait for selection the semen.

Results Only two of 23 announcements assessed took into account some reproductive trait in the selection criteria for buy the semen. In these cases the trait was daughter fertility. This is an important trait, considering that the last two or three decades, the selection and genetic improvement were focused primarily in the ones involving milk production and conformation, while performance for traits like longevity, fertility and disease resistance tended to decrease (Thaler Neto, 2006). Several studies show that reproductive efficiency is less when it is associated with an increase in production rates often related to physiological stress, such as a negative energy balance that occurs intensively in highly productive animals. However, there are many factors that can influence the reproduction, such as adaptation to climate, diet quality and quantity and quality of forage available over the year (Silva et al., 1998). Despite reproductive traits presents generally low heritability, it becomes important to select in order to improve these traits, due to reproductive problems found in the farms. Those, increase the calving interval, age at first calving and the number of semen doses required for conception, among others factors, which worsened after intense selection for high productive animals.

Conclusion The reproductive traits, despite the great importance for dairy cattle production, are not properly taken into account by public agencies of Western Santa Catarina State in the choice of semen to use in the herds.

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Correlation study between reproduction and conformation traits in Holstein bulls with genetic evaluation available in Brazil

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Implications Historically in Brazil, little attention tends to be given to type and reproduction traits when selection is made. This study helps to clarify the importance of considering the association with these traits during the selection.

Introduction It is necessary to assess the association between reproduction and conformation traits to analyse how they behave when the selection is made. Particularly in Brazil, greater attention was given to production traits, due to the payment system focused on amount of milk. Less importance is given to type and conformation traits and rarely reproduction is included in the selection index. The knowledge of the correlation between these traits enables us to develop appropriate breeding plans for genetic improvement. In this context, the aim of this study was to estimate the correlation coefficient between the Predicted Transmitting Ability (PTAs) of reproduction traits with conformation traits in Holstein bulls with genetic evaluation available for commercialization in Brazil.

Material and methods Records of 385 Holstein bulls in Brazilian companies supplying semen in 2008 were located on the company's websites. Currently, the most of productive dairy cows in Brazil are female calves of the bulls in this study. The genetic evaluation of these bulls to the traits described were located and tabulated from the Dairy Bulls website (http://www.dairybulls.com). Statistical analysis of the correlation between PTAs of reproduction traits (calving ease, productive life, pregnancy rate and stillbirths) and linear traits (stature, strength, body depth, angulosity, rump angle, rump width, rear legs side view, rear legs rear view, foot angle, feet and legs, rear udder width, udder cleft, udder depth, front and rear teat placement, teat length) and composition of udder, body, feet and legs and dairy form were performed by correlation analysis using the software Minitab® (Minitab version 14, 2004, State College, PA) using the tukey test, considering 5% of significance level.

Results In this study the majority of the correlations between the reproduction traits and conformation are low (< 0.30) and not significantly different from zero, as well as was verified by Wall *et al.* (2005). The linear traits: stature (-0.21), strength (-0.25), body depth (-0.30) and dairy form (-0.20) and the conformation traits: body composition (-0.25) and dairy form (-0.23) showed negative correlation with productive life. These traits also had negative correlations with pregnancy rate. Selection for higher dairy form may be indirectly selecting for cows that are more inclined to reproductive diseases (Rogers *et al.*, 1999). Animals with high angulosity tend to present less pregnancy rates of which the correlation found was -0.18. In this study feet and legs obtained positive correlation with productive life (0.13~0.26). Pérez-Cabal and Alenda (2002) suggest in their study that introducing feet and legs as a linear trait in a type-production index is a way to improve functional longevity as was also found in the current study. Udder conformation and depth also showed positive correlation with productive life (0.24 and 0.30 respectivelly). With this, selecting to feet and legs in addition to udder conformation, can increase the cow longevity. The correlation between body composition and calving ease was positive (0.21). Stature, strength, body depth and rump width are also positively correlated to calving ease (0.17; 0.22; 0.21; 0.19) but attention should be given on selection for these traits because they had negative correlations with productive life and pregnancy rate. There are no significant results correlating rump angle and fertility (P>0.05) such as shown by Wall *et al.* (2005). There was a low correlation regarding to calving ease and rump width (0.19), but a negative correlation between these traits was found by Cue *et al.* (1990). It was also observed that a bad teat placement negatively affects calving ease (-0.12 ~ -0.16) and increase stillbirths (-0.14 ~ -0.17).

Conclusion The selection to conformation traits can lead to increases in the herd reproductive performance.

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A comparison of the concentrations of pro-inflammatory cytokines in the uterine flush of dairy cows with or without endometritis

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Implications The greater concentrations of TNFα, IL-1β and IL-6 in the uterine flush during early lactation strongly suggest the existence of clinical or subclinical endometritis, further reflecting the severity of uterine diseases in dairy cows.

Introduction The first defense barricade coping with pathogenic bacteria is polymorphonuclear cells, which are mobilized through the release of inflammatory cytokines from immune cells. TNFα, IL-1β, IL-6, IL-8 and IL-10 have been known as the inflammatory cytokines associated with bovine endometritis. Endometrial biopsy or cytobrush techniques have been used for detection of these cytokines in uterine tissue via the expression of mRNA. Otherwise, serum cytokine levels were determined in cows with endometritis. However, detection of the inflammatory cytokines from uterine flushes has not been reported in dairy cows with endometritis. Therefore, this study compared the changes in concentrations of TNFα, IL-1β and IL-6 concentrations at weeks 4, 6 and 8 *postpartum* in uterine flushes of cows with clinical endometritis,or subclinical endometritis and normal cows.

Material and methods Forty-eight Holstein dairy cows (2.3 ± 1.5 lactations), were used for this study. Uterine flush samples were collected for analyzing the TNFα, IL-1β and IL-6 levels. The samples were taken at 4, 6 and 8 weeks *postpartum* by flushing of a uterine horn with 20 ml of physiological saline solution using a 2-way Foley catheter. The collected uterine flush (13.7 ± 5.7 ml) were centrifuged (750 g for 10 min at 4°C) and the supernatant was transferred into 2 mL microcentrifuge tubes, and stored frozen at -20°C until analysis. TNFα, IL-1β and IL-6 concentrations in the uterine flush were measured with ELISA. Clinical endometritis was diagnosed by retrieving vaginal discharge at week 4 *postpartum*, then cows showing mucopurulent discharge were regarded as having clinical endometritis (LeBlanc et al., 2002). Subclinical endometritis was diagnosed using uterine cytology. Subclinical endometritis was defined when the proportion of neutrophils was > 10% at week 6 *postpartum* (Sheldon et al., 2006). Thus, forty-eight cows in the present study were divided into three groups: clinical endometritis group (n = 20), subclinical endometritis group (n = 15) and normal group (n = 13). Repeated measures ANOVA was used to compare the changes in the mean TNFα, IL-1β and IL-6 concentrations of the uterine flush at weeks 4, 6 and 8 *postpartum* among the three groups.

Results The TNFα concentrations of the uterine flush in the clinical endometritis group (422.0 ± 63.6 pg/ml) was greater ($P < 0.01$) than in the normal (141.9 ± 14.6 pg/ml) and subclinical endometritis (162.6 ± 28.9 pg/ml) groups at week 4 *postpartum*, while they did not differ ($P > 0.05$) at weeks 6 or 8 *postpartum* among groups. The IL-1β concentrations of the uterine flush did significantly differ ($P < 0.05$) among the three groups at weeks 4, 6 and 8 *postpartum*, respectively: 2,418.2 ± 423.1, 1,471.7 ± 428.5 and 564.4 ± 310.3 pg/ml in the clinical endometritis group, 229.0 ± 98.0, 191.7 ± 138.8 and 34.7 ± 7.8 pg/ml in the subclinical endometritis group, and 47.4 ± 20.1, 50.1 ± 25.0 and 19.9 ± 3.5 pg/ml in the normal group. The IL-6 concentrations of the uterine flush differed ($P < 0.05$) at weeks 4 and 6 *postpartum* among the groups: 4,221.7 ± 843.8 and 2,412.9 ± 848.7 pg/ml in the clinical endometritis group, 1,261.6 ± 506.4 and 477.7 ± 164.2 pg/ml in the subclinical endometritis group, and 235.2 ± 64.9 and 144.1 ± 32.7 pg/ml in the normal group, respectively. However, the IL-6 concentrations in the clinical endometritis (1,697.5 ± 913.7 pg/ml) and subclinical endometritis (657.8 ± 244.8 pg/ml) groups was greater ($P \leq 0.01$) than in the normal group (176.2 ± 53.7 pg/ml) at week 8 *postpartum*.

Conclusion Concentrations of the TNFα, IL-1β and IL-6 in the uterine flush of dairy cows with clinical or subclinical endometritis were greater than in normal cows, among which the IL-1β showed the most uniform results.

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Survey of genomic management practices of United States' dairy producers

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Implications Producer satisfaction level with dairy cattle reproduction was dependent on reported annual 21-day pregnancy rate; however, the use of genomic testing was independent of reported pregnancy rate.

Introduction Maternal fertility is a lowly heritable polygenic trait. Our collaborative group has embarked on a 5-year research and extension project to develop novel genetic fertility markers in heifers and lactating cows, determine effects of single nucleotide polymorphisms on daughter pregnancy rate and embryo development, and understand gene pathways associated with daughter pregnancy rate, fertilization and embryo development. The objective of the survey was to examine current producer opinions, awareness, and management strategies regarding genomics to provide a foundation for the extension component of the project.

Material and methods Between 6 August 2013 and 5 February 2014, United States' dairy producers were encouraged to complete the on-line survey through electronic methods including newsletters and websites (Dairy Alert, Dairy Agenda Today, Dairy Business Communications, Hoard's Dairyman), state newsletters (California, Utah, Idaho, Washington, New Mexico, Virginia, and Florida), and via magazines (Progressive Dairyman, Western Dairy Business). Frequencies were tabulated for binary and categorical variables (Caraviello et al., 2006). Chi-square analyses were performed using MiniTab.

Results Dairy producers submitted 334 surveys. Nearly all producers (99%) remarked they had read articles and seen advertisements regarding genomic testing, while 67% mentioned the use of genomic testing in their herd. The most prevalent reasons, in descending order of importance, for use of genomic testing were to 1) aid in the selection of genetically superior animals for internal use or marketing, 2) verify parentage, 3) aid in the decision-making process in mating heifers, and 4) aid in the decision-making process to "cull" or "keep" heifers. Among respondents who had not used genomic testing, 63% remarked they had considered genomic testing. Satisfaction level with reproduction was dependent on reported annual 21-day pregnancy rate (Chi-Sq = 38.9; $P < 0.05$; Table 1). The use of genomic testing was independent of reported pregnancy rate (Chi-Sq = 3.1; $P > 0.05$; Table 2). Annual average 21-d pregnancy rate for lactating cows in the U.S. is 14 – 18% (Niles et al., 2001; Moeller et al., 2010).

Table 1 Satisfaction level with reproduction relative to annual 21-day pregnancy rates for lactating cows[1]

	Response				
21- day pregnancy rate	No	% of row total	Yes	% of row total	Count Total
Equal to or less than 15%	13	77	4	23	17
16 – 18%	48	68	23	32	71
19-21%	41	41	58	59	99
Equal to or greater than 22%	38	28	100	72	138

[1]325 respondents answered both questions

Table 2 Use of genomic testing relative to annual 21-day pregnancy rates for lactating cows[1]

	Response				
21-day pregnancy rate	No	% of row total	Yes	% of row total	Count Total
Equal to or less than 15%	8	47	9	53	17
16 – 18%	19	27	52	73	71
19-21%	31	31	68	69	99
Equal to or greater than 22%	48	35	90	65	138

[1]325 respondents answered both questions

Conclusions The use of genomic testing appears to be independent of reported pregnancy rate. Table 2 provides evidence that United States' dairy producers, across a spectrum of reported annual 21-day pregnancy rates, have used genomic testing. A key challenge identified in this survey is to develop a broad-based extension program capable of providing 1) decision-making tools to encourage producers who have considered, but not yet used genomic testing to do so, and 2) advanced support to further enhance the use of genomic testing among those already using the technology.

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Effect of timing of artificial insemination and ovulation in relation to onset of standing heat on pregnancy rate in dairy buffaloes

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Implications The artificial insemination using frozen semen should be done at either 12 or 24 hours after the onset of standing heat in order to achieve the maximum fertility in buffaloes.

Introduction Buffalo is main dairy animal in many Asian countries including Pakistan. Fertility with frozen semen is compromised in buffaloes (Andrabi et al., 2001). Factors responsible for low conception rate with artificial insemination (AI) in buffaloes include poor estrus expression, semen quality, body condition, insemination technique and hygienic conditions (Anzar et al., 2003). The Single most important factor in achieving high conception rate in buffalo can be the proper timing of AI relative to the ovulation. Timing of ovulation has not been well studied in buffalo. This has been reported to be delayed in buffalo compared to cows in relation to onset of standing estrus (Kanai and Shimuzi, 1983; Roelofs et al., 2004). Perhaps this is why the AM-PM rule which was originally developed in cow (Trimberger, 1948) when applied in buffalo resulted in lowered fertility. Therefore, the objective of the present study was to determine timing of ovulation and best time of AI after the onset of standing heat on pregnancy rate in dairy buffalo.

Materials and methods The experiment was conducted at Livestock Experimental Station of Buffalo Research Institute, Pattoki, District Kasur, Pakistan during the breeding season (Sep-Dec) of 2012. Eighty five multiparous, suckled Nili-Ravi buffaloes, 4-6 years of age and 350-450 kg of weight were managed and fed under optimal conditions. Estrus detection was carried out twice daily (6:00 a.m. and 6:00 p.m.) for a period of 30 minutes, using a penile deviated teaser bull. The buffaloes coming in estrus spontaneously, were assigned randomly to be artificially inseminated either at 0 (n=30), 12 (n=27) and 24 (n=28) hours after the onset of standing estrus. Frozen thawed semen from a single bull (Semen Production Unit, Qadirabad, Sahiwal, Pakistan) of known fertility was used by a single experienced technician. Transrectal ultrasonography (Honda; Model: HS-1500; 7.5 MHz) was performed in order to determine the timing of ovulation at twelve hourly intervals, in a subset (n=25) of buffaloes beginning from the onset of standing heat. Ovulation was considered when the dominant follicle present in the last scan disappeared in the subsequent one. Pregnancy status of the experimental buffaloes was determined using transrectal ultrasonography, 35-40 days after artificial insemination. Pregnancy per AI was calculated as: [(number of buffaloes pregnant divided by number of buffalo inseminated) x 100)] and was compared amongst different insemination times using χ^2-test for several proportion tests (SPSS, version 10:00). A probability level of ($P<0.05$) was considered significant.

Results The mean overall timing of ovulation in Nili-Ravi buffalo was 35.3±0.21 hours from the onset of standing heat. Mean overall size of follicle just before ovulation was 15.05±1.16 mm. Highest pregnancy rate, 53% (15/28) was observed in buffaloes inseminated at 24 h, followed by 37% (10/27) at 12 h. Lowest pregnancy rate, 26% (8/30) was seen in buffaloes inseminated at 0 h. The overall pregnancy rate was 40% (34/85). The pregnancy rate did not differ significantly ($P > 0.05$) between 0 h and 12 h groups. Similarly, pregnancy rate did not differ ($P > 0.05$) between buffaloes of 12 h and 24 h. However, pregnancy rate differed significantly ($P < 0.05$) between buffaloes inseminated at 0 h and 24 h.

Conclusion It is concluded that timing of ovulation is about 35 h and maximum pregnancy rate are achieved when bred either at 12 or 24 hours after the onset of heat in buffaloes.

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Elevated non-esterified fatty acid concentrations during bovine oocyte maturation influences DNA methylation in blastocysts

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Implications Oocyte maturation under elevated concentrations of non-esterified fatty acids leads to changes in DNA methylation patterns of genes related to metabolic and cell survival pathways. These epigenetic alterations may contribute to a deviating foetal growth and altered postnatal health.

Introduction Metabolic stress associated with negative energy balance (NEB) is a risk factor for decreased fertility in high producing dairy cattle. Non-esterified fatty acids (NEFA) are implicated in this pathogenesis as they are present in the oocyte's micro-environment during final maturation. Elevated NEFAs hamper the granulosa cell function (steroidogenesis) and jeopardize oocyte maturation leading to reduced developmental competence (Leroy *et al.*, 2005). More specifically, Van Hoeck *et al.* (2013) showed that oocyte maturation in the presence of elevated NEFA concentrations is subject to alterations in the gene transcription profile of the resultant blastocysts. Even the expression of genes essential for the establishment of epigenetic markers (*DNMT3A*, *HIST1H2BN*) was altered in response to elevated NEFA levels during final oocyte maturation. Changes in these epigenetic markers may induce pertinent changes in gene expression: effects which only become evident after birth or even in later life. In this study, we hypothesized that the maternal lipolytic conditions around conception (such as NEB) alter the epigenetic regulation. Therefore, we matured oocytes under physiological and elevated NEFA concentrations and used the EmbryoGENE Bovine methylation microarray to define the DNA methylation profile in the resultant blastocysts.

Material and methods *In vitro* embryo production was performed as described by Van Hoeck *et al.* (2011). A total of 1039 oocytes were collected and equally assigned to 2 treatments during 24 hours of *in vitro* maturation (4 replicates): 1) physiological NEFA conditions (mixture of 23 µM palmitic acid (PA), 28 µM stearic acid (SA) and 21 µM oleic acid (OA)) and 2) elevated NEFA concentrations as under lipolytic conditions (mixture of 150 µM PA, 75 µM SA and 200 µM OA). Cleavage and blastocyst rates were determined at 48 hours and 7.5 days after fertilization, respectively. A total of 80 day 7.5 blastocysts were used for DNA and RNA extraction and the DNA was analysed for methylation patterns using the EmbryoGENE Bovine methylation microarray platform. Images were generated with the Tecan PowerScanner microarray scanner and converted into intensity data files using the ArrayPro 6.4 Analyzer software. Data were normalized and fitted to a linear model using the Limma package (Linear Models for Microarray Data). The significance threshold was set to an absolute fold-change greater than 1.5 and a p-value smaller or equal to 0.05. Differences in affected pathways were identified using Ingenuity Pathway Analysis.

Results The microarray data reveal a significant difference in DNA methylation of 210 genes of which 119 genes were hyper- and 91 genes were hypomethylated in the blastocysts originating from oocytes matured under elevated NEFA concentrations compared to the blastocysts originating from oocytes matured under physiological NEFA concentrations. Significant differences in methylation patterns were present in genes involved in the process of apoptosis with almost the same number of genes being hyper- and hypomethylated. Similar distribution patterns between hyper- and hypomethylated genes were observed in the lipid metabolism pathways. Also methylation status of genes involved in the regulation of inflammation was affected suggesting a decrease of inflammatory response as there were more genes hypermethylated than hypomethylated. The methylation pattern of genes related to gene transcription was changed with more hypermethylated than hypomethylated genes. At the same time, hypermethylation of a gene important for histone methylation (*JARID2*) was observed. Alterations in this gene can lead to changes in other epigenetic markers. Also, significant changes in methylation of genes associated with diabetes and obesity in humans were observed.

Conclusion Maturation of oocytes in the presence of elevated NEFA concentrations causes changes in the methylation patterns of different genes in the resultant blastocysts. These epigenetic changes could influence the embryonic development and even the onset of disease in later life. Evaluation of the gene-expression of the differently methylated genes through qRT-PCR is necessary to confirm these results.

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The effect of lameness on oestrus activity in high yielding Holstein-Friesian dairy cattle with access to pasture

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Implications Step counts for lame and non-lame dairy cattle increased during oestrus. Lame cattle had significantly reduced lying times compared with non-lame cows.

Introduction Increasing global demand for dairy products has subsequently increased production goals from dairy farmers. Modern high yielding Holstein Friesian dairy cows have been reported to have reduced intensity of oestrus, thus making fertility an ongoing issue in the dairy sector. Additionally, lameness affects dairy herds worldwide (Green *et al.,* 2014), which may further exacerbate fertility issues, and could adversely affect animal welfare and increase costs through premature culling and additional veterinary treatment. Lameness has been reported to affect oestrous behaviour, however the incidence of oestrus remains unaffected (Walker *et al.,* 2010). Additionally, Blackie *et al.* (2011) reported that lame cows increase their lying time when compared with non-lame cows. This could directly influence oestrus behaviour as the time available to express oestrus is reduced. The aim of the study was to assess the effect of lameness on oestrous activity in dairy cattle.

Material and methods A herd of approximately 100 high yielding Holstein Friesians (10975 kg) with access to pasture were studied from February to October 2013. Following calving cows were assessed for lameness weekly (Flower and Weary 2006). Cows were scored after milking on a flat concrete alley. Each cow was given a locomotion score from normal to severely lame (locomotion scores 1 to 5, respectively). Cows were chosen based on their locomotion score, and their current reproductive stage. Cows were enrolled in the study from approximately 20-35 DIM, parities ranged from 3-5. Lame cows had a mean locomotion score and parity of 3.2 (±SE 0.1) and 3.9 (±0.2), respectively. Non-lame cows had a mean locomotion score and parity of 1.9 (±0.2) and 3.4 (±0.3), respectively. Cows were matched based on parity and were fitted with IceQube® Sensors (IceRobotics Ltd). A total of 32 oestrous events were recorded from n=9 lame, and n=8 non-lame cows. Data were downloaded wirelessly, and analysed (GenStat, 16th edition) by ANOVA with LSD

Results There was no significant difference in step counts before, during or after oestrus in lame and non-lame dairy cattle (Figure 1). There was a significant difference ($p<0.001$) in lying times between lame and non-lame dairy cattle (Figure 2). Lame cows had a mean lying time of 6.9 hrs (±0.1), and non-lame cows had a mean lying time of 8.7 hrs (±0.1).

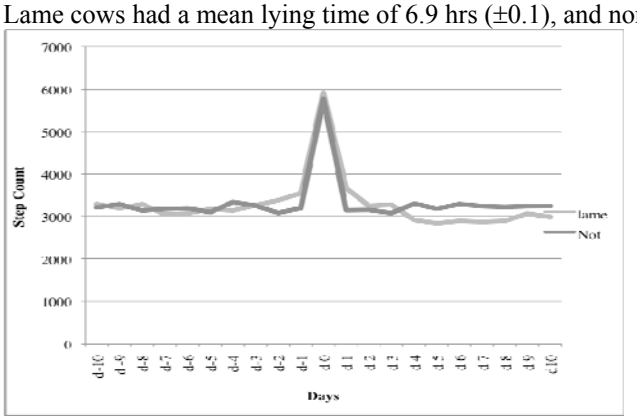

Figure 1 Step count for lame and non-lame dairy cattle before, on the day of, and after oestrus

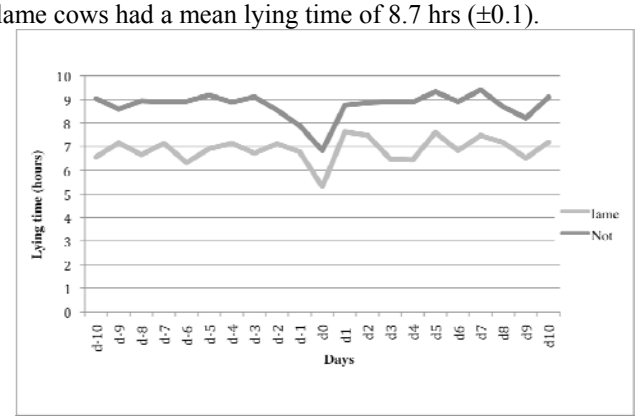

Figure 2 Lying times (hrs) for lame and non-lame dairy cattle before, on the day of, and after oestrus

Conclusion Lame cows from this herd had no significant difference in step counts. This could be due to the small difference in locomotion score between the lame and non-lame cows (1.3), or that pasture reduced the effects of lameness, thus not influencing step counts. However, despite the small difference lame cows had significantly reduced lying times when compared with non-lame herd mates.

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The effect of bovine placenta extract application on the postnatal complications, insemination efficiency and milk yield of the first lactation period Russian Black Pied cows

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Implications The procedure for prevention and treatment of the postnatal complications based on application of tissue extract produced from bovine placenta has been developed.

Introduction The maintenance of reproduction performance of dairy cows has a great importance in modern milk production (Remppis et al., 2011). In Russia, the level of postnatal diseases reaches, in some cases, 85-90 %. The different natural and artificial substances are used to improve the calving and lactation capacity. The artificially synthesized substances (hormones and antibiotics) contain the limited number of compounds and thus couldn't provide with full effect on the reproduction performance (Zheng, 2012). The endocrine function of placenta, during pregnancy, is one of the most important events that influence both the future of the calving and the next lactation (Cotor, 2011). The other hand the tissue preparations may cause allergic reactions and infections. Also their biological activity dependents on the tissue origin and tissue preparation procedure. The aim of this study was to evaluate the efficiency of bovine placenta extract application for correction of reproduction performance of the first-calf cows.

Material and methods Placenta extract was performed by mechanical homogenization of bovine placenta tissue and subsequent extraction in a microwave oven. The extract was diluted in distilled water supplemented with 20% thymol and stored at $+4^0$C until application. Each batch of extract was tested on rats to standardize the activity. 20 ml of placenta extract was subcutaneously injected alternatively on the right and left side of the neck Black Pied heifers using four different treatments. The 1st group heifers received 4 injections - 60±2, 30±2, 15±2days before the expected calving date and on the day of calving, the heifers of the 2nd group were injected 3 times - 30±2, 15±2days before the expected calving date and on the day of calving, the animals of the 3rd group received 2 injections - 15±2days before the expected calving date and on the day of calving and the 4th group heifers were not treated. The effect of the placenta extract was evaluated by the rate of postnatal complications (retention of placenta, endometritis of different etiology), by the efficiency of the subsequent insemination and milk yield for the first 100days of lactation period. Statistical data were analysed using the chi-square test.

Results It was shown that the injection of bovine placenta extract to heifers before and after calving decreased the rate of animals with the retention of placenta and endometritis of different etiology compared to the untreated animals ($P < 0.05$) (Table 1). The positive effect of placenta extract was not dependent on the number of performed injections. The average pregnancy rates after first insemination in the treated animals was 51.7±6.4 % that was on 11.7 % higher than non-treated cows. The highest milk yield for the first 100days of lactation period was in the 2nd group cows – 2854.3±65.83kg that was on 9.4 % higher ($P < 0.01$) than non-treated cows. The administration of the bovine placenta extract to the first-calf cows was made without any adverse reactions, and no risk towards the animal's health.

Table 1 Effect of bovine placenta extracts on the rate of postnatal complications heifers

Group	Number of injections	Number of animals	Number of animals with pathology of *postpartum* period	
			n	%
Group 1	4	20	6	30,0±10,25*
Group 2	3	20	7	35,0±10,67*
Group 3	2	20	7	35,0±10,67*
Group 4	-	20	13	65,0±10,67

*$P < 0.05$: significant differences between groups 1-3 and group 4.

Conclusion The injections of the bovine placenta extract to the heifers before and after calving decrease the rate of postnatal diseases and increase the milk yield of Black Pied cows.

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Comparison of two oestradiol esters administered with previously used CIDR for oestrous synchronization in buffalo cows during the low breeding season

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Implications Oestrous synchronization (ES) with UCIDR in conjunction with oestradiol benzoate (EB) and oestradiol dipropionate (EDP) with FTAI (fixed time AI) may be used successfully in buffalo cows during low breeding season.

Introduction Buffalo cows (*Bubalus bubalis*) are the best dairy animals of Pakistan accounting for 62 % of total milk production. They tend to be sexually more active from September-October. During the low breeding season, buffaloes exhibit a high incidence of silent oestrus or anoestrus. This leads to a longer calving interval. In order to enhance reproduction in low breeding season ES protocols have been tried including PG, GnRH and Progesterone (P4). P4 based ES in the form of CIDR devices are getting popular in buffaloes. Previously used CIDR (UCIDR) has also been reported of the same value as that of new CIDR (NCIDR) (Colazo et al. 2004). Injection of EB after CIDR removal resulted in improved fertility in anoestrus buffaloes. Stilbestrol (EDP) is another oestradiol ester that is easily available locally at a very low cost. This study was carried out to compare the effect of EB and EDP in conjunction with UCIDR on the oestrus intensity/ response, size of ovulatory follicle, different intervals to ovulation, ovulation and pregnancy rate in buffaloes.

Materials and methods The study was carried out at Buffalo Research Institute, Pattoki, Kasur, Pakistan during February-May 2013. Sixty adult buffaloes having normal reproductive tracts were randomly selected at about 60 days *postpartum*. To reduce the cost, UCIDRs were used in this study following disinfection procedure as described by Naseer et al. 2011. All the animals were randomly divided into four groups, having 15 buffaloes in each group. Animals in group 1 (control group) received no treatment while animals of group 2 (UCIDR), 3 (UCIDR+EB) and 4 (UCIDR+EDP) received UCIDR (Eazi-Breed, Pfizer) for 7 days with an injection of $PGF_{2\alpha}$ (Dalmazine, FATRO, Italy) at day 6 after UCIDR insertion. FTAI was performed 48 hours after UCIDR removal with 12 hours interval. Additionally, animals of group 3 and 4 received an injection of EB (0.4 mg/ 4ml; Sigma, St. Louis, MO, USA) or EDP (1mg/ 3ml; Stilbestrol; Star Laboratories, Lahore) i/m at 24 hours after UCIDR removal, respectively. Ovaries of experimental animals were monitored twice daily through ultrasonography (Honda, Model: HS-1500; 7.5 MHz) at 12 hour intervals from day 8 to ovulation to check the size of ovulatory follicle and time of ovulation. Oestrus detection (ED) was carried out twice daily at 12 AM and 6.30 AM. Inspite of ED, FTAI was performed 48 hours after UCIDR removal. Pregnancy was confirmed by ultrasonography on day 40. Statistical analysis of % values was carried out through Chi square while other values were analysed under CRD through one way ANOVA using software SPSS (Version 20.0.1 In., Chicago, IL, USA).

Results Oestrus response and pregnancy rate amongst all the groups differed non-significantly ($P > 0.05$) while ovulation rate in group 3 was significantly ($P < 0.05$) higher compared with groups 1 & 2. Size of ovulatory follicle was significantly larger ($P < 0.001$) in buffaloes of group 1 as compared with those of other groups. The intervals from PG administration, CIDR removal and EB/EDP injection to ovulation amongst all the groups were non-significant ($P > 0.05$) (Table 1 & 2).

Table 1 Oestrus response, ovulation and pregnancy rate in different groups of buffalo cows

Groups	1 (Control)	2 (UCIDR)	3 (UCIDR+EB)	4 (UCIDR+EDP)	P-value
Oestrus response (%)	60 (9/15)[a]	80 (12/15)[a]	93 (14/15)[a]	87 (13/15)[a]	0.12
Ovulation rate (%)	53 (8/15)[a]	60 (9/15)[ac]	93 (14/15)[b]	80 (12/15)[bc]	0.05
Pregnancy rate (%)	20 (3/15)[a]	26 (4/15)[a]	33 (5/15)[a]	20 (3/15)[a]	0.76

Table 2 Size of ovulatory follicles and different intervals to ovulation in ovulating buffaloes (Mean ± SE)

Groups	1 (Control)	2 (UCIDR)	3 (UCIDR+EB)	4 (UCIDR+EDP)	P-value
Size of ovulatory follicle (mm)	15.4 ± 0.5[b]	12.9 ± 0.2[a]	12.9 ± 0.4[a]	12.4 ± 0.4[a]	0.001
Interval from $PGF_{2\alpha}$ inj to ovulation (h)	-----	86.0 ± 2.0[a]	79.7 ± 4.1[a]	78.0 ± 2.1[a]	0.17
Interval from CIDR removal to ovulation (h)	-----	62.0 ± 2.0[a]	55.7 ± 4.1[a]	54.0 ± 2.9[a]	0.17
Interval from EB/ EDP inj to ovulation (h)	-----	38.0 ± 2.0[a]	31.7 ± 4.1[a]	30.0 ± 2.9[a]	0.17
Interval from standing heat to ovulation (h)	46.5 ± 11.9[b]	32.0 ± 2.0[a]	24.8 ± 4.4[a]	22.0 ± 3.7[a]	0.001

Conclusion UCIDR may be used with EB/ EDP with same efficacy in buffalo cows during low breeding season.

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Trends and research in genetic aspects of fertility of dairy cattle in New Zealand

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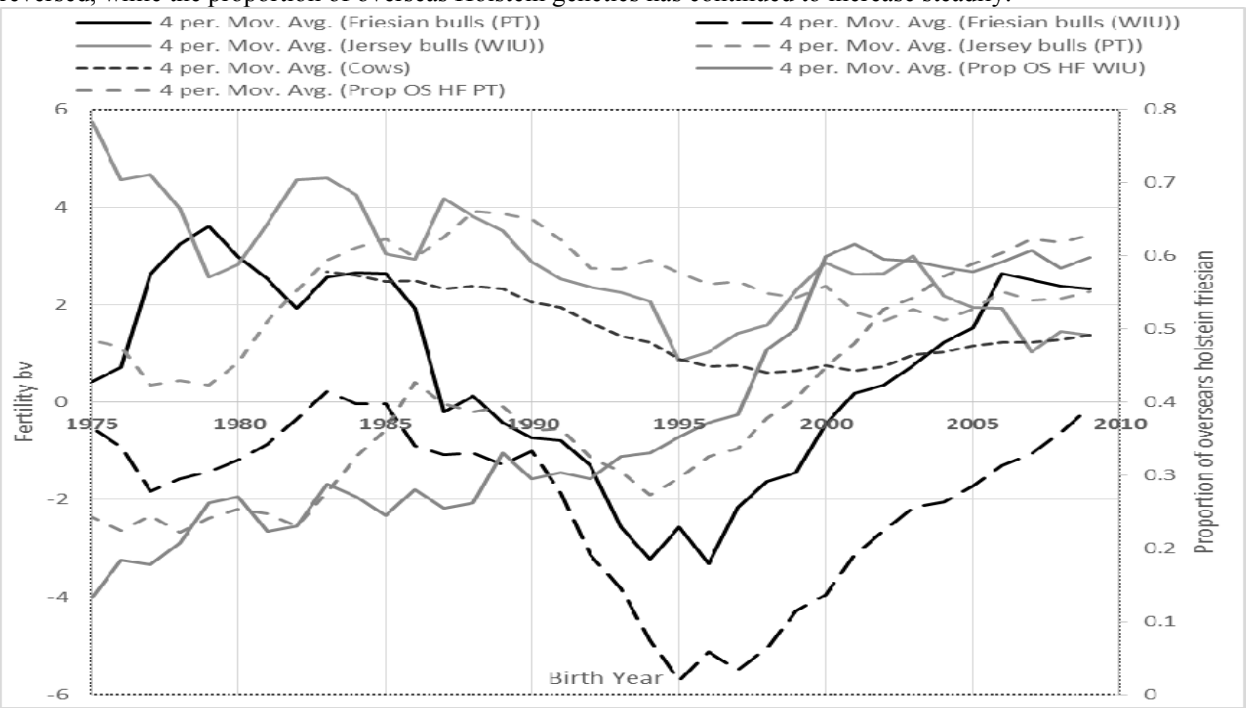
Implications Good fertility is a high priority trait for the New Zealand dairy cow because the seasonal nature of the NZ farming system requires a 365 day average calving interval. Some management interventions used to achieve this, such as inductions, are unlikely to be available in the future. This means that NZ must make further genetic improvements in cattle fertility.

Introduction In the past, fertility was not a main focus when selecting NZ dairy sires and this meant that there was a decline in fertility of dairy cows during the 1990's, especially in Friesian cattle, that the industry is still recovering from. This communication will focus on the industry genetic trends, the history of genetic evaluation and the current fertility research in fertility in NZ.

Material and methods Fertility breeding values for dairy bulls and cows were collected from NZAEL data and collated by birth year and breed. Sires that had greater than 1000 daughters herd tested for production were classified as industry bulls and sires that had between 40 and 100 herd tested daughters were classified as progeny tested young (PT) bulls that did not progress to widespread industry use. The mean of the data was weighted by the number of herd tested daughters for each sire.

Results Fertility breeding values (bvs) for PT bulls for both Friesians and Jerseys were consistently below those of the industry bulls, suggesting that bulls were rejected for industry use on the grounds of poor daughter fertility. From the mid 1980's to the mid 1990's fertility bvs declined in sires, especially in Friesians. After the introduction of the fertility bv to the national genetic evaluation (the breeding worth), fertility bvs have been improving. Friesian fertility has recovered from its nadir of the mid 1990's, however there is still a significant disparity between the fertility of sire proving and industry sires indicating that more emphasis on fertility when selecting among PT Friesian bulls for widespread industry use. Jersey fertility has yet to recover to the levels achieved in the 1980's and is in danger of being surpassed by Friesian industry sires. At a population level, the New Zealand Friesian bulls initially declined in fertility as the proportion of Overseas Holstein genetics in bulls increased, but over the past two decades, calculated genetic trends suggest the trend in fertility has been reversed, while the proportion of overseas Holstein genetics has continued to increase steadily.



Conclusion Dairy cattle fertility is improving in NZ. However, phenotypically, there are still many herds with unacceptably high rates of infertility and a number of research initiatives are under way to improve the rate of genetic progress in fertility.

Acknowledgements

References Data was obtained from the NZAEL website (http://www.nzael.co.nz/animal-data/bull-search)

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Failure to Imprint: Hypomethylation trends at imprinted loci in oocytes recovered from *postpartum* dairy cows

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Implications The metabolic environment of the *postpartum* dairy cow adversely affects ovarian function as evidenced by aberrant methylation patterns in maternally imprinted genes in oocytes recovered by ovum pick from cows 20 to 120 days post partum

Introduction During the period of post-partum negative energy balance in high yielding dairy cows, key molecular and morphological events that occur during oocyte and follicle growth, such as genomic imprinting in the growing oocyte, may be detrimentally affected by this altered ovarian environment. Maternally imprinted genes are methylated during the growth stage of oogenesis, whereby the differentially methylated region (DMR) on the maternal allele is fully methylated (the paternal copy, originating from the sperm, is fully unmethylated). Correct imprinting is crucial for placental function and regulation of foetal growth; aberrant methylation marks at imprinted loci may contribute to early embryonic loss.

Material and methods Using repeated ovum pick-up (OPU), oocytes and follicular fluid samples were recovered at regular intervals from multi-parous dairy cows between days 20 and 120 post-calving. Immature oocytes were pooled on a per cow basis at three time points *postpartum*: early (≤ 45 dpp), mid (46-80 dpp) and late (>80 dpp). DNA methylation levels at imprinted genes were analysed using pyrosequencing assays. Individual follicular fluid samples were assigned to the same time points *postpartum* and the amino and fatty acid profiles of the follicular fluid was analysed using gas chromatography mass spectrometry. Analysis of variance (ANOVA) and post hoc Bonferroni's test were performed using SPSS (SPSS, version 20.0) to identify any significant (P<0.05) metabolites between the three groups. Principal component analysis (PCA) was performed using SIMCA-P+ (Umetrics) to identify separation between the datasets and outliers as appropriate. In a complimentary study, cumulus oocyte complexes were *in vitro* matured in the presence of high concentrations of non-esterified fatty acids with or without methyl-donor S-adenosylmethionine (SAM) supplementation. The proportion of oocytes that cleaved and reached the blastocyst stage were analysed using Mixed procedure of SAS (SAS Institute). Differences among means were determined by F-tests using Type III sums of squares. The PDIFF option (SAS Institute) and the Tukey test were applied to evaluate pairwise comparisons between means.

Results Average methylation values were determined by pyrosequencing analysis of the following maternally methylated imprinted gene DMRs, *SNRPN*, *MEST*, *IGF2R*, *PLAGL1*, *PEG3* and the paternally methylated imprinted gene, *H19*. For fully-grown oocyte samples recovered ≤ 45 dpp, methylation values of 1.4% (*PEG3*), 32% (*PLAGL1*) and 3% (*SNRPN*) were recorded. In oocytes isolated from animals 46-80 dpp, methylation values were as follows; 83.4 - 96.7% (*PEG3*); 29.6% (*MEST*); 83.2 - 87.4% (*IGF2R*) and 91.9% (*PLAGL1*), while values of 1.4% and 11.6% (*MEST*), 1.5% (*PLAGL1*) and 76.7% (*SNRPN*) were recorded in oocytes isolated >80 dpp. Interestingly, there was a high incidence (7 out of 10) of hypermethylation of the *H19* DMR at the early, compared with later recovery dates (mid) 0 out of 9, and (late) 1 out of 4. Collectively, imprinted gene DMR methylation was highly variable in oocytes recovered within the first 45 days post calving, or from 85 days to 120 days. Pyrosequencing analysis revealed loss of DNA methylation at the *PLAGL1* DMR in oocytes, following *in vitro* maturation (IVM) in the presence of elevated NEFAs and SAM. In addition, *in vitro* embryo development was significantly reduced following IVM in the presence of elevated NEFAs and SAM (p<0.05). Metabolomic analysis of *postpartum* follicular fluid samples identified 27 fatty acids, of which, the abundance of six were significantly different (p<0.05) across time; pentadecanoic acid and tricosanoic acid concentrations increased with increasing dpp, whereas concentrations of docosahexanoic acid diminished in parallel to dpp. Concentrations of eicosatrienoic, *cis*-11-eicosenoic and heneicosanoic acid decreased between the early and mid group then increased between the mid and late group. Eighteen amino acids were identified, of which, valine, leuine, creatinine, phenylalanine, ornithine and glutamine concentrations altered significantly over time pp (p<0.05). The abundance of both valine and leucine increased significantly in follicular fluid with increasing dpp, whereas concentrations of creatinine and ornithine decreased. However, PCA of both amino acid and fatty acid concentrations in the three dpp groups (early, mid & late) indicated that there was no distinct trend in the data (amino acid, R2=0.45 and fatty acid, R2=0.34).

Conclusion
The establishment and maintenance of DNA methylation imprints at the DMRs of maternally methylated imprinted genes was compromised in the oocytes of post-partum dairy cows. This failure to imprint may contribute to early embryonic mortality observed in high yielding cattle.

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Associations between polymorphisms in five candidate genes, identified from transcriptional profiling of uterine endometrial tissue, in Holstein-Friesian bulls with either high or low genetic merit for calving interval

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Implications Associations between novel SNPs and fertility traits in cattle were identified in candidate genes which had previously been shown to be differentially expressed in uterine endometrial tissue between heifers of either high or low fertility status. These may represent polymorphisms with direct effects on uterine gene expression and thereby early embryo development and warrant further investigation.

Introduction Reproductive performance of dairy cows has declined by approximately 0.5% to 1% per annum, in recent decades. The majority of reproductive wastage is attributed to early embryonic loss, for which 70-80% occurs in the first two weeks post AI (Diskin *et al.* 2008). Our group have identified distinct gene expression profiles from uterine endometrial tissue, collected on Day 7 or Day 14 of the oestrous cycle, from heifers ranked as either high or low fertility (Killeen *et al.* 2014). DNA sequences of 10 of these candidate fertility genes (*ALB*, *BMPR2*, *COL4A3*, *COL4A4*, *CYP4F2*, *DAP*, *FST*, *GALNT6*, *PCCB* and *SFRP1*) were characterised using DNA isolated from performance-tested sires displaying differential genetic merit for fertility (Killeen *et al.* 2011). The hypothesis of the current study was that these candidate genes may harbour polymorphisms associated with fertility in cattle. The objective of the current study was to ascertain if the mutations identified as segregating at significantly different frequencies between the high and low genetic merit DNA pools associated with fertility performance using a cohort of 848 Holstein-Friesian bulls with daughter progeny in Ireland.

Material and methods DNA from semen straws of 150 Holstein-Friesian (HF) bulls, 75 each with either high (HCIV) or low (LCIV) genetic merit for calving interval were pooled. Methodology on the generation of the DNA pools, target enrichment and high throughput sequencing data analysis, including allele frequency estimation, has been described (Mullen *et al.* 2012). Of the 233 variants identified (Killeen *et al.* 2011), 63 polymorphisms were selected for genotyping based both on allele frequency differentials (> 2-fold) between the EBV divergent DNA pools and/or potential effects on gene function. Genotyping of a selection of the identified SNPs, was carried out using iPLEX-MassArray technologies (Sequenom Inc.) across 848 Holstein-Friesian bulls with daughter progeny in Ireland. The association between each SNP and fertility related performance traits (functional survival, calving interval, gestation length, calving difficulty and calf mortality) were individually quantified using weighted mixed models, accounting for pedigree structure.

Results Nominal associations ($P<0.05$) were observed between 25 SNPs in five genes (*FST*, *GALNT6*, *ALB*, *DAP* and *SFRP1*) with functional survival, calving interval, gestation length, calving difficulty and calf mortality, in the panel of 848 sires. Of particular interest, five SNPs in *GALNT6*, four of which result in non synonymous substitutions, were associated ($P<0.05$) with at least one of the five fertility traits. For example, The C allele of a putatively novel SNP in *GALNT6*, GALNT6 SNP9, was associated with a reduction in calving interval of 1.1 days ($P<0.02$) and 0.74% increase in functional survival ($P<0.01$). In addition, another putatively novel SNP in *FST* (FSTSNP1), was associated ($P<0.05$) with decreased gestation length of 0.34 days, decreased calving interval of 0.78 days and increased functional survival of 0.35%. The C and A alleles of two SNPs in *DAP* were associated ($P<0.05$) with increased calf mortality of 0.24% and 0.25%, respectively.

Conclusion The use of transcriptional data on targeted tissues is a promising approach to identify candidate genes potentially harbouring causal mutations affecting performance in livestock. We have identified associations between polymorphisms in genes originally identified from global uterine transcriptional profiles and fertility performance in cattle. Whether the observed effects are causal i.e. directly affecting uterine gene expression or due to other polymorphisms in linkage remains to be determined. Future work will include validation of the SNP associations in independent populations of cattle and *in vitro* analysis to ascertain causality.

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Insulin during oocyte maturation alters gene expression and development rates in *in vitro* produced day 8 bovine blastocysts

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Implications Bovine oocytes treated with insulin during maturation resulted in lower blastocyst rates on day 8 (BC8). Analysis of microarray data of 120 *in vitro* blastocysts day 8 (BC8) showed differential expression of 202 transcripts in the high and 142 transcripts in the low insulin treatment group in comparison with an untreated control group.

Introduction Metabolic imbalance impairs fertility and is common in the dairy cow during the transition period while variation in insulin concentrations in serum and follicular fluid can occur. Insulin is a key metabolic hormone and deviation from physiological insulin levels in serum and follicular fluid might impair oocyte maturation and early embryonic development as described by Adamiak *et al*. (2005). The aim of the present experiment was to study the effects of elevated levels of insulin during oocyte maturation on subsequent *in vitro* embryonic development and gene expression in Day 8 blastocysts.

Material and methods Abattoir-derived oocytes (n=1900) were separated into 3 groups and *in vitro* matured for 22 h with different insulin concentrations (H: High 10µg/ml; L: Low 0.1 µg/ml and Z: Zero, control). Subsequent fertilisation and culture of zygotes were performed according to standardized procedures described by Abraham *et al.* (2012). Cleavage (2 cells or above/ immature oocytes) and Day 8 blastocyst rates (Day 8 blastocysts/ immature oocytes) were recorded. From the total number of Day 8 blastocysts (n=390), those deriving from the first 9 batches were stained for morphological evaluation whereas blastocysts from the last 8 batches were washed twice in PBS and frozen in -80°C in view of gene expression studies. Blastocysts (n=120) were pooled in groups of 10 and total RNA was extracted by parallel gDNA-and total RNA-extraction (AllPrepDNA/RNA micro kit, cat no. 80284, Qiagen). All samples (4 biological replicates /treatment group) resulted in RIN–values above 7.5. RNA-amplification, cDNA-synthesis, purification and labelling was performed and 825ng Cy3 and Cy5- labelled linearly amplified aRNA was hybridized on the Agilent-manufactured EmbryoGENE-slides in a 2-colors dye swap design. Slides were scanned with PowerScanner (Tecan, Mannedorf, Switzerland). In search for the differentially expressed genes between the control and the insulin treated groups an empirical Bayes moderated t-test was then applied, using the 'limma' package in R.
Significant transcripts were defined as having a 1.5 fold-change difference between treatment and control and $P<0.05$. Statistical analysis of developmental rates was performed using SAS software, version 9.1, with ANOVA tests using procedure GLM following arc sin Vp transformation. Multiple comparisons post ANOVA were performed by using the Scheffe and the contrast option.

Results Cleavage rates did not differ between the groups. BC8-rates did not differ between the high and the low insulin groups but both were slightly lower than in controls (H: 17.8±1.8%; L: 17.8±1.8%; Z: 22.6±1.8%; H+L vs Z, $p<0.03$).
Analysis of the gene expression patterns between the insulin treated versus the control group revealed significant differential expression of 202 transcripts in the H and 142 transcripts in the L group. 104 of the differentially expressed transcripts were found in both insulin groups. Most transcripts were up-regulated by insulin treatment. In the H group, only 8 genes were down- and 194 genes were up-regulated. In the L group, 4 were down- and 138 up-regulated. Validation of data by q-PCR and further analyses of the transcriptome data has yet to be done.

Conclusion The impaired blastocyst developmental rates in the insulin-treated groups indicate a negative effect of supra-physiological insulin concentrations during oocyte maturation. Further investigations and analyses of the genes and pathways involved and of the possible dose effect of insulin need to be performed to approach the molecular mechanisms behind the impaired development.

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Effect of lactation on the metabolic profile of dairy cattle

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Implications A comparison of the metabolic profiles of lactating cows, dry cows (calved and not milked) and dairy heifers indicates significant interactions between parturition and lactation which may confound reproductive parameter results observed in lactating versus heifer models. The most appropriate comparison to use is a dry cow and this is reflected in the metabolic profiles in circulation.

Introduction Infertility in dairy cattle is a multifactorial problem that may be attributable to compromised oocyte or embryo quality and/or a suboptimal reproductive tract environment or a combination of both. The physiological changes associated with milk production impact on circulating metabolites during the early post partum period and likely play a role in poor reproductive efficiency. Using *postpartum* dairy cows that were either dried off immediately at calving (i.e., never milked) or were milked twice daily as well as nulliparous dairy heifers, the aim of this study was to characterise the effect of lactation on the metabolic profiles of dairy cattle.

Material and methods Forty in-calf dairy cows (first lactation) and 20 age matched dairy heifers with similar Economic Breeding Indicies were enrolled onto the study. From two weeks prior to calving body weight (BW) and body condition score (BCS) were recorded and blood samples were collected twice weekly up to 84 days post partum (dpp). Immediately after calving, half of the cows were dried off (i.e., never milked) while the other half entered the milking herd and were milked twice daily. Serum samples were analysed for markers of metabolic status including non-esterified fatty acids (NEFA), β-hydroxy butyrate (BHB), glucose, insulin and Insulin like growth factor 1(IGF1). Pre-calving all animals were fed 30 kg grass silage/head/day with pregnant heifers getting an additional 3 kg concentrates/head/day. Post calving, dry cows had ad-lib access to grass silage plus 4 kg concentrates/day, while lactating cows received 24 kg maize silage/16 kg grass silage plus 7 kg concentrates. Data were analysed using a repeated measures mixed models ANOVA (PROC MIXED) in SAS incorporating terms for treatment group, sample time and their interaction, as appropriate.

Results The BW and BCS of dry and lactating cows were similar throughout the study period and were significantly lower than nulliparous heifers ($P<0.0001$) throughout the period. Heifers had significantly higher serum insulin concentrations in the pre-partum period and up to 10 dpp at which time concentrations in dry cows were similar. Insulin concentrations were lowest ($P<0.0001$) in lactating cows throughout. Similarly, circulating IGF1 concentrations were higher in heifers than both post-partum groups throughout the period of study. IGF1 concentrations decreased in the two weeks prior to calving and remained low throughout the period in lactating cows, while in dry cows concentrations increased gradually from calving to d84 pp. Glucose concentrations spiked at calving in the dry and lactating groups. Post-calving, concentrations were similar in dry cows and heifers and were significantly lower ($P<0.0001$) in lactating cows from day 7 pp onwards. Concentrations of NEFA were basal (<0.2 mmol/L) in heifers throughout the study. Both lactating and non-lactating post-partum cows exhibited an elevation in NEFA beginning 10 days prior to calving. In the dry group, NEFA concentrations returned to basal levels rapidly after calving (by day 10 pp) while in lactating cows concentrations were significantly elevated until approximately day 42. Lactation significantly increased BHB concentrations post partum which remained high compared to with the dry cows and heifers ($P<0.0001$).

Conclusion Lactation significantly alters the metabolic profile of dairy cows post partum, in a manner that is independent of the effects of parturition (compared with the dry group) and their interactions (heifer group). Given this significant difference in order to understand the effect of lactation, without the confounding effects of parturition, the metabolic profile of calved cows that were dried off was a more appropriate model than dairy heifers when examining various reproductive parameters.

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Optimising the storage temperature and sperm concentration of liquid stored bull sperm

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Implications The use of liquid stored bull semen is cost efficient and best utilises elite young sires in grass based production systems which have a short breeding season.

Introduction Currently in Ireland, liquid bull sperm contains approximately 5 x 10^6 sperm per insemination dose, is stored at unregulated ambient temperature and is inseminated within 2.5 days of collection due to concerns of declining fertility. We have recently shown that lower sperm concentrations reduce oxidative stress in liquid stored bull sperm (Murphy et al, 2013) and this coupled with storage of sperm at 5°C, which has been shown to reduce metabolic activity, may be a viable way to extend the fertile lifespan of liquid stored bull sperm. The objective of this study was to evaluate the best storage temperature for liquid stored bull sperm stored in a range of sperm concentrations.

Material and methods Semen was collected from Holstein Friesian, Norwegian Red, Limousin and Aberdeen Angus bulls at a commercial AI centre (5 collections with 3 bulls per collection). Sperm concentration was assessed and each ejaculate was diluted in a 5% egg yolk Caprogen diluent to a final concentration of 10 (T_{10}), 5 (T_5), 1 (T_1) and 0.5 ($T_{0.5}$) x 10^6 sperm per 0.25 mL straw. For experiment 1, post packaging, straws were stored at either 15°C or 5°C and assessed *in vitro* on Days 0, 1, 3 and 5 post-collection (Day 0 = day of collection). On each assessment day, all sperm concentrations and temperature groups were evaluated for total motility (Phase-contrast microscope; 400X), viability, acrosomal status, DNA fragmentation and osmotic resistance (ORT; T_{10} only) by flow cytometry. For experiment 2, sperm was collected from 6 bulls (Holstein Freisian, Aberdeen Angus and Hereford), diluted and packaged, as above, and stored at either 15°C, 5°C or fluctuated between 15 and 5°C (Flux) to mimic fluctuation from day to night time temperatures. For Flux, straws were submerged in a waterproof container, and incubated at either 15°C during the day or at 5°C at night, to allow a gradual temperature fluctuation. Sperm were assessed *in vitro* on Days 0, 1, 3 and 5 for total motility, viability and ORT. Flow cytometry plots were gated and 10,000 events were analysed. Data were examined for normality, homogeneity of variance and analysed using the repeated measures procedure in SPSS, with the exception of ORT which was analysed using the univariate procedure. The model included the main effects of day, sperm concentration, temperature, and their interactions.

Results In experiment 1, storage at 15°C had greater motility post Day 1 in comparison with 5°C ($P<0.001$). There was no effect of sperm concentration on motility at 15°C, however, at 5°C, $T_{0.5}$ had lower motility than both T_{10} and T_5 ($P<0.05$). There was no effect of day, sperm concentration or temperature on viability, acrosomal status or DNA fragmentation. The ORT of sperm declined with duration of storage and this decline was greater at 15°C than at 5°C ($P<0.001$). In experiment 2, the 15°C and Flux treatments maintained a greater motility post Day 3 ($P<0.01$; Fig. 1). For Flux, T_{10} and T_5 maintained a greater motility than $T_{0.5}$ ($P<0.01$). There was no effect of sperm concentration or temperature on viability. The 5°C and Flux treatment maintained a greater ORT post Day 3 in comparison with the 15°C treatment ($P<0.001$).

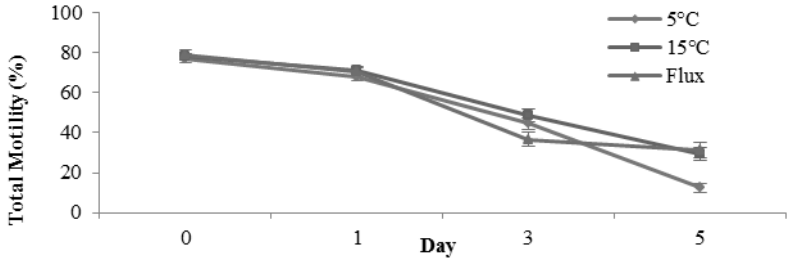

Figure 1 Experiment 2; the percentage of progressively motile sperm (mean of all sperm concentrations) at 15°C, 5°C, and Flux. Vertical bars represent ± s.e.m.

Conclusion When stored at 15°C the motility of liquid stored bull sperm was retained for longer compared to storage at 5°C. Despite this, sperm stored at 15°C had reduced membrane function as is evident by reduced osmotic resistance. However, storing sperm under controlled conditions, fluctuating between 15°C and 5°C, was not detrimental to either motility or osmotic resistance, compared to storage at 15°C or 5°C, respectively.

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Effect of equine chorionic gonadotrophin administration on corpus luteum development, circulating progesterone concentrations and embryo development in cattle

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Implications Elevating circulating progesterone concentrations in early pregnancy can promote early embryo development, maternal recognition of pregnancy and reduce embryo mortality.

Introduction Insertion of a progesterone device post oestrus increases circulating progesterone and advances conceptus elongation (Carter et al., 2008). However, in a proportion of animals the CL is compromised resulting in a shortened cycle (O'Hara et al., 2013). The aim of this study was to examine the effect of a single i.m. injection of equine chorionic gonadotrophin (eCG) on Day 3 post oestrus on corpus luteum (CL) development, circulating progesterone concentrations and conceptus development in cattle.

Material and methods In Experiment 1, the oestrous cycles of crossbred beef heifers (n=60) were synchronised using a 7-day progesterone releasing intravaginal device (PRID Delta®, CEVA) with administration of a prostaglandin F2α analog (Enzaprost®, 5 mL equivalent to 25 mg dinoprost, CEVA) given on the day before PRID Delta removal. Heifers were checked for signs of oestrus four times per day commencing 30 h after PRID Delta withdrawal. Only those seen in standing oestrus (n=48) were randomly assigned to one of five treatment groups: (1) Control: saline, or a single i.m. injection of eCG on Day 3 at a concentration of (2) 250 IU eCG (3) 500 IU eCG (4) 750 IU eCG or (5) 1000 IU eCG. Daily jugular blood samples were collected to determine circulating progesterone and estradiol concentrations. Daily transrectal ultrasound scanning was carried out to monitor follicular growth and CL development.

Experiment 2 was designed to test the hypothesis that the luteotrophic effects of a single i.m. injection of eCG on Day 3 seen in Experiment 1 would abrogate the negative effects of exogenous progesterone on CL survival. The oestrous cycles of n=40 heifers were synchronised as before. Those seen in standing oestrus (n=32) were randomly assigned to receive (i) a PRID delta from Day 3 to 5 or (ii) a PRID from Day 3 to 5 plus a single injection of 750 IU eCG on Day 3. *In vitro* produced blastocysts (n=10 per recipient) were transferred on Day 7. Daily blood samples were collected from Day 2 to 14 to determine progesterone concentrations. All heifers were slaughtered on Day 14 to assess embryo development.

Results In Experiment 1, administration of eCG resulted in increased luteal tissue area and circulating progesterone and estradiol concentrations compared to controls in a dose-dependent manner from approximately Day 9 onwards. The effect was most marked with 750 or 1000 IU eCG. In Experiment 2, administration of eCG at the time of PRID Delta insertion reduced the number of short cycles (6.3%, 1/16 v 31.3%, 5/16) and increased mean luteal tissue weight ($P=0.02$) and area ($P=0.07$) at slaughter. Insertion of a PRID Delta on Day 3 resulted in an elevation ($P<0.05$) in serum progesterone which remained elevated until removal on Day 5. Administration of eCG at the time of PRID Delta insertion resulted in higher progesterone ($P<0.05$) from Day 10 onwards compared to the PRID Delta only group. Overall recovery rate (conceptuses recovered as a proportion of embryos transferred) was 44.1% (141/320) and was not different between groups. Administration of eCG at the time of PRID Delta insertion did not affect conceptus dimensions compared to the PRID Delta only treatment.

Conclusion Results show that a single injection of eCG on Day 3 increased CL size and progesterone concentrations and when given in conjunction with a progesterone-releasing device appeared to reduce the number of short cycles, presumably due to its luteotrophic nature. The implications of the elevated estradiol concentrations for embryo quality require further study.

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The use of a cowside lateral flow milk progesterone test to aid reproductive management in a dairy herd

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Implications The availability of a rapid, convenient cowside test for milk progesterone represents a major advance in the ability to manage dairy cow fertility. As a result, wastage due to poor fertility is reduced, with a concurrent improvement in animal welfare and farm profitability

Introduction On farm milk progesterone measurement has proved to be an effective aid to reproductive management in dairy cows, but its uptake has been limited by the inconvenient nature of available ELISA based methods. A new cowside dipstick method, based on lateral flow technology, has the potential to give results within five minutes using a one step dipstick method. The present study was one of a series designed to evaluate the effectiveness and potential economic benefits of the 'P4 Rapid' (Ridgeway Science) milk progesterone kit for reproductive management.

Material and methods Milk samples were taken from individual cows, before applying the milking machine, in a 400 cow dairy herd (a) if suspected heat signs from visual observation or a movement detector system were recorded and/or (b) 19 to 22 days after insemination. Progesterone levels were measured and recorded on farm using the P4 Rapid test. The samples were then sent to Ridgeway Science for analysis using the Ridgeway quantitative ELISA milk progesterone assay. Using results from the on farm test, the following decisions were made (for both (a) and (b) above): (a) cows were inseminated if a low progesterone value confirmed the absence of an active corpus luteum or (b) cows were not inseminated if a high level indicated that the cow was in the luteal phase or pregnant. Cows with high levels of progesterone 19-22 days after insemination were put forward for veterinary pregnancy diagnosis in line with existing farm policy.

Results A total of 196 milk samples were analysed on farm. Ninety-six putative oestrus events were confirmed, resulting in 52 pregnancies. Seventeen of these were lost before manual PD, giving a final pregnancy rate of 36%, an improvement over the herd's previous performance. Three oestrus events were confirmed after cows had suffered fetal loss, allowing for a repeat service in each case thus saving at least three weeks in the final calving interval. Insemination at the wrong time was prevented in one cow in mid cycle, saving the cost of semen and removing the risk of causing infection. Insemination was prevented in sixteen pregnant cows, although six of these subsequently lost their fetus. Approximately 50% of inseminations during pregnancy cause abortions (Ball and Peters, 2004), so that the test potentially saved 5 pregnancies. Overall, action based on the P4 Rapid information led to considerable economic benefits, which will be discussed in detail in the presentation**.**

Conclusion The on-farm use of lateral flow dipstick tests for cows' milk progesterone can provide a convenient and highly cost effective means of aiding fertility management,. It is particularly effective in the prevention of insemination at the wrong time, e.g. during pregnancy, which can potentially have very serious consequences.

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Breeding soundness evaluation of 40 bulls with reduced reproductive performance: a retrospective study

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Implications Male fertility is an important factor in bovine reproduction given that a single bull is generally bred to numerous females. This is especially true in herds where AI is not used and in herds where a bull is used at the end of the AI breeding season as a so called sweeper, as a bull that is infertile or sub-fertile can have detrimental effects on the reproductive efficiency of such herds (Parkinson, 2004).

Introduction A full breeding soundness evaluation (BSE) of a bull is essential before he is introduced to the main breeding herd. It consists of a general clinical examination, a genital tract examination of both the external (prepuce, penis, testes, scrotal circumference) as well as internal genitalia (seminal vesicles, prostate, penis) followed by a semen quality evaluation of which the main components are progressive motility and morphology of the sperm cells (Kastelic and Thundathil, 2008).

Material and methods Forty bulls aged between 1.2 and 5.8 years were presented to our clinic with a history of reduced reproductive performance. All bulls were used for natural service. All bulls were examined for general health after which examination of the reproductive tract took place, consisting of inspection and palpation of the external genitalia and measurement of the scrotal circumference using the Reliabull Scrotal Tape (Lane Manufacturing Ltd. Denver, Co, USA). A rectal examination was then performed to examine the internal genitalia. Semen was obtained twice 45 minutes apart from all bulls by use of an electro-ejaculator (Pulsator IV, Lane Manufacturing Ltd. Denver, Co, USA). Once electro-ejaculation was commenced all excretions were collected. Following collection, the ejaculate was assessed immediately for the following parameters: volume (mls), colour (1= almost clear, 2=skim milk, 3=milk), concentration ($x10^6$) and linear motility (1-5). Percentage (%) live sperm and % sperm with normal morphology were determined after a sperm smear was stained with eosin–nigrosin and evaluated under the microscope (x1000 magnification under oil). The combination of the semen evaluation data and the clinical examination results were used to classify the bulls as fertile (normal scrotal circumference, no abnormalities of reproductive tract, normal concentration of sperm with >50% live sperm with good (3-5) motility and >70% normal) or infertile (no sperm or <50% live and poor (1) motility and <50% normal). Bulls with results between these 2 classes were classified as sub-fertile. A chi-square test was used to assess the unadjusted relationship between fertility classification and colour and motility. Age at examination, scrotal circumference, semen volume, concentration, percentage live sperm and percentage normal sperm, all continuous variables were divided into categorical variables based on quartiles. The chi-square test was then used to assess the unadjusted relationship between fertility classification and each variable.

Results Characteristics of the 40 bulls examined (Mean ± SEM) are displayed in Table 1. Colour (P<0.001), motility (P=0.05), concentration (P<0.001) and percentage live sperm (P=0.002) were significantly associated with fertility classification. Of those classified as infertile (n=13), motile sperm were reported in only three of the animals examined.

Table 1

	n	Age (y)	SC (cm)	Semen vol (ml)	Semen Conc $x10^6$	% live sperm	% normal sperm
Fertile	17	2.6±0.32	39.5±0.78	7.5±0.69	177±24.2	75.5±3.05	80.2±1.45
Subfertile	10	2.2±0.15	40.1±0.90	7.5±0.93	123±23.9	40.4±4.63	75.5±2.16
Infertile	13	2.8±0.46	36.7±1.48	6.0±0.61	208±79.8	25.6±5.84	52.6±6.34

Conclusion In this retrospective study we found that when performing a full breeding soundness examination in bulls, the colour, motility, concentration and % live sperm in the semen sample were significantly associated with fertility classification.

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Effect of physiological / disease status on the response of *post partum* dairy cows to synchronisation of oestrus using a CIDR device

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Implications Dairy cows suffer from immunosuppression around the time of parturition which can decrease the fertility of the animal.

Introduction Progesterone treatments are used to increased submission rates in *postpartum* dairy cows, however, in many cases the protocol is used as a blanket therapy for all cows without regard for physiological/disease state (Lane et al., 2008). The objective was to identify physiological/disease classes of cows that do not respond well to progesterone synchronisation.

Material and methods This study was conducted using multiparous (N=250) and primiparous (N=152) cows from five commercial spring-calving dairy herds in the Leinster region (Ireland). All animals were housed indoors within a free-stall barn during their 60 day dry period before calving. After parturition they remained indoors within a free-stall barn until turn out to pasture in spring (varied from early February to April across the farms) with the exception of one herd that remained indoors throughout the study. Breeds represented were Holstein (n=100), Holstein-Friesian (n=203) and Crossbreds (n=99). The distribution of breeds on individual farms ranged from primarily Holstein-Friesian to primarily crossbreds. All cows enrolled on the trial were evaluated at seven time points around calving. At each sample / evaluation time locomotion score (LM), body condition score (BCS), rectal temperature as a measure of overall health and vaginal mucus score (MS) as measurement of uterine health were recorded. In addition, blood samples for metabolic parameters were collected. Insulin-like growth factor-1 (IGF-1), Glutathione peroxidase ((GpX) as an estimate of the Selenium status), Beta Hydroxybutyrate (BHB), Non-esterified fatty acids (NEFA), Urea, Calcium (Ca), Magnesium (Mg) and Phosphorus (P) were analysed. Animals were grouped into classifications according to their status: negative energy balance (NEB), clinical lameness, uterine infection (UI), anovulatory anoestrus (AA), high somatic cell counts (SCC) and as healthy (H). All animals received an eight day CIDR protocol which included GnRH at insertion and PGF2α the day before removal. Response to the protocol was determined by visual observation of oestrous behaviour with the aid of tail paint. Conception rate was determined by ultrasonography on day's 32-35 post A.I. Metabolic parameters were classified as continuous variables and were analysed using mixed models, with farm, cow identity, lactation, response and calving status as fixed effects, and time point as a repeated effect using SAS (Version9.3). The classifications were classified as binary variables as well as the response to the synchrony protocol and the pregnancy status. The logit of the probability of a positive outcome for these variables was evaluated using logistic regression.

Results Animals without UI were 1.9 times more likely to respond and 2 times more likely to be confirmed in calf than those with UI ($P \leq 0.05$). There was no relationship between NEB and clinical lameness in the visual oestrous response, but both conditions were associated with reduced ($P \leq 0.05$) conception rates. Dairy cows in AA responded positively to the protocol ($P < 0.05$). Results showed the more crossovers between groups, the lower the probability of oestrus and conception ($P < 0.05$). High levels of GpX concentrations had a positive effect on conception rates while high levels of NEFA and BHB had a negative effect on the oestrous response ($P < 0.05$).

Conclusion In conclusion, the disease and physiological status of dairy cows determined the response to progesterone based synchronisation. The more disease/physiological problems cows had the lower the response and conception rates and were therefore not ideal candidates for synchronisation. Both anoestrus and healthy dairy cows were good responders to progesterone-based synchronisation treatment.

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Genetic associations between detailed reproductive traits and milk production in dairy cows

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Implications Results from this study highlight generally antagonistic genetic relationships between milk production traits and detailed reproductive traits derived from ultrasound examination of the reproductive tract substantiating the known genetic antagonisms between milk production and traditional fertility traits.

Introduction Clear evidence exists of unfavourable genetic correlations between milk production and traditional reproductive traits such as calving interval, number of services and pregnancy rate. However, little is known of the genetic associations between milk production and detailed reproductive traits, especially those related to reproductive tract health. The objective, therefore, of the current study was to quantify the genetic correlations between milk production traits and detailed reproductive traits derived from ultrasound examination of the reproductive tract in Irish dairy cows.

Material and methods Individual cow milk production and reproductive tract ultrasound records were available from the Irish Cattle Breeding Federation database between the years 2008 and 2012. The milk production traits included 305-day lactation yield of milk, fat and protein, as well as milk fat concentration and protein concentration. All ultrasound measurements were performed by a single company. A measurement of the right ovary, left ovary and a cross section of the uterine horns were used to classify the reproductive status of the animal. Four detailed reproductive traits were defined: resumption of cyclicity (CYCLE) defined by the presence of a corpus luteum (CL) on the ovaries (CYCLE=1) or not (CYCLE=0) at time of examination, multiple ovulation (MULTI) defined as two or more CLs present (MULTI=1) in cyclic animals at time of examination, cystic structures (CYST) defined as the presence of a cystic structures >30mm on one or both ovaries at the time of examination and uterine score (UTERUS) defined as an ordinal trait on a scale of 1 (normal uterine tone, <2mm fluid) to 4 (poor uterine tone, >60mm of fluid) based on the amount of fluid and structure of the uterine wall. Contemporary groups were defined as herd-year-season of calving and contemporary groups with <5 animals for milk production traits were discarded. A random sample of the data for production traits was retained. After all edits, 113,812 production records from 106,733 cows and 65,394 ultrasound records from 34,827 cows remained. Variance components were estimated using repeatability animal models and genetic correlations were estimated using a series of bivariate repeatability sire models. Fixed effects included in the models for all traits were parity, heterosis, recombination loss of the dam and contemporary group; stage of lactation (0-14, 15-39, 40-84, 85-149, 150-300 and >300 days *postpartum*) at time of examination was also included as a fixed effect for all detailed reproductive traits. Animal/sire and permanent environmental effects were included as random effects.

Results Heritability estimates (standard errors in parenthesis) of the detailed fertility traits where 0.06(0.010), 0.01(0.009), 0.04(0.014) and 0.02(0.006) for CYCLE, CYST, MULTI and UTERUS, respectively. Heritability estimates for the milk production traits ranged from 0.21 to 0.37. The genetic correlations between detailed fertility traits and production traits are in Table 1. As genetic merit for yield increased the ability to resume cyclicity *postpartum* reduced, uterine health deteriorated, and the incidence of multiple ovulations increased. Increased genetic merit for fat and protein concentration was associated with more normal uterine tone and less uterine fluid. Additionally, increased in genetic merit for greater protein concentration was associated with a greater ability to resume cyclicity *postpartum*. CYST was not genetically associated with any of the milk production traits.

Table1 Genetic correlations (standard errors in parenthesis) between detailed fertility traits and milk production traits.

	Milk kg	Fat kg	Protein Kg	Fat %	Protein %
CYCLE	-0.40(0.08)	-0.29(0.09)	-0.38(0.09)	0.06(0.09)	0.28(0.08)
CYST	0.06(0.05)	0.02(0.04)	0.05(0.05)	-0.01(0.04)	-0.01(0.03)
MULTI	0.31(0.12)	0.26(0.13)	0.12(0.13)	-0.15(0.13)	-0.11(0.17)
UTERUS	0.14(0.07)	0.16(0.08)	0.11(0.07)	-0.11(0.06)	-0.10(0.06)

Conclusion The antagonistic genetic correlation that existed between the milk production traits and the detailed reproductive traits in the present study corroborates the known genetic antagonism between milk and traditional fertility traits. This provides evidence that selection for production traits impacts unfavourably on most characteristics of the underlying reproductive tract.

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Superovulation rates and embryo yields for heritage breed cattle and commercial dairy cattle

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Implications Heritage breed cattle (HB) can be successfully superovulated using standard protocols resulting in an acceptable yield of transferable embryos (TE).

Introduction The commercial dairy cattle industry has been experiencing a trend toward decreasing fertility rates as a result of intensive genetic selection for fluid milk production (McGinnis, 2009). HB have not undergone this same selection pressure and in the future may become a valuable reservoir of lost genetic material, including genes that regulate fertility. SVF Foundation (SVF) was established in 2002 to create an *ex situ* repository for germplasm, including cryopreserved embryos from heritage breeds of livestock (Roof *et al*, 2012). Because of genetic differences there was concern HB might respond differently to standard superovulatory protocols. The objective of this study was to compare superovulatory response (total ova/coll) of HB cows at SVF to the response of Holstein–Friesian cows (HF) superovulated under similar conditions.

Material and methods The 83 heritage breed cows in this study represented 9 different breeds and ranged in age from 1.5 to 14 years (avg. 4.6 yrs). HB were sourced from small, privately held herds across the U.S., with an effort made to obtain representation of maximum genetic diversity for each population. HB were superovulated an average of 3.7 times each (range 1-7) and embryos collected at SVF Foundation in Newport, RI. Embryos from HF were collected on privately owned dairy farms within 200 kilometres of Newport, RI. All cattle received injections of follicle stimulating hormone (FSH) every 12 hours over a four day period. Treatments began at day 10 of the oestrous cycle following observation of a reference heat or alternatively after four days of progesterone supplementation using an intravaginal insert (CIDR,® Zoetis) and an injection of GnRH 2 days later. Prostaglandin was administered with the 7th and 8th FSH injections and CIDR®s (when used) were removed at the 7th injection of FSH. Superovulation protocols were adjusted based on donor age, parity, stage of lactation, weight and prior response to FSH. All cattle were artificially inseminated at the onset of oestrus and again 12 and 24 hours later. Non-surgical embryo collection was performed on all cattle by the same veterinarian on day 7 post-insemination, and embryos were handled and graded using criteria set by the International Embryo Transfer Society (IETS). As expected for these two complex data sets, data were not normally distributed (Shapiro-Wilk, $p<0.05$). Therefore a non-parametric Mann-Whitney Rank Sum test was used to assess differences in superovulation response and yield of transferrable embryos (TE) between HF and HB populations.

Results HB were successfully superovulated using standard protocols (Table 1). Comparing HF to HB (aggregate), the average total ova per collection was not statistically different ($p>0.05$). However, average TE per collection was significantly different ($p = 0.003$) between the two populations.

Table 1 HB and HF superovulation and embryo collection

HB	Dams (n)	Sires (n)	Collections (n)	Total ova/coll	TE/coll	Discards/coll
Candienne	11	10	40	12.5	5.8	6.7
Dutch Belted	10	15	45	10.6	4.8	5.8
Kerry	13	14	45	10.9	3.7	7.2
Milking Devon	16	12	63	7.1	3.9	3.2
Milking Shorthorn	7	11	26	5.9	3.3	2.6
Pineywoods	3	9	14	7	3.2	3.8
Randall Lineback	8	10	29	10.5	4.1	6.4
Red Poll	4	5	8	9.4	3.9	5.5
White Park	11	12	36	12.9	3.9	9
HB (aggregate)	83	98	306	9.9	4.2	5.7
HF	156	118	347	9.2	5.3	3.9

Conclusion The use of standard superovulation protocols in this study did not produce different outcomes in total ova/collection in HB. This supports future use of such protocols for harvest of embryos from non-commercial cattle breeds as part of a large scale effort aimed at preserving germplasm from heritage breeds of cattle.

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Expression of IL-1ß and its receptor IL R tI in bovine endometrium and embryos during early development

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Implications Using an *in vitro-in vivo* model we showed that the IL-1 system could play a role in the bovine embryo-maternal crosstalk during the period of blastocyst formation. Furthermore we detected for the first time Interleukin-1 receptor type I (IL-1R tI) in bovine blastocysts. This information might contribute to improve the *in vitro* culture of bovine embryos.

Introduction The IL-1 system, detected in mammalian embryos and in the reproductive tract, has been suggested as the initiator of conceptus-uterine cross talk during pregnancy (Lindhard et al. 2002; Ross et al. 2003). In cattle, IL-1ß has been detected in the endometrium throughout the estrous cycle (Paula-Lopes et al. 1999) and in embryos and uterine fluid during early preimplantation development (Muñoz *et al*. 2012) but no information is available about the Interleukin-1 receptor type I (IL-1R tI). In this work we analysed the expression of IL-1ß and IL-1R tI as well as their co-localization in bovine embryos and endometrium during the early development (between day 5 and day 8).

Material and methods Synchronized recipients were transferred with multiple *in vitro* produced morulae on day 5 after *in vitro* fertilization (ET; N=5). On day 8, animals were slaughtered and their embryos recovered by uterine flushing. Control animals were sham transferred (ST; N=6), and control embryos were entirely cultured *in vitro* until day 8. Localization of IL-1ß and IL-1R tI in endometrium and embryos were performed by immunohistochemistry and confocal microscopy. Endometrial protein expression differences were evaluated by western blot (WB). Data were analyzed using the GLM procedure of SAS Version 9.2 and REGWQ test for means.

Results In the endometrium, IL-1ß and IL-1R tI were highly co-localized in the glandular and luminal epithelial cells, whereas IL-1R tI localized only to blood vessel walls and myometrium. No differences in staining patterns were found between ET and ST animals. WB analyses of IL-1ß showed a major band of 35kDa and a smaller band of 17 kDa corresponding to the reported pro-form and matured forms of IL- 1ß. IL-1ß pro-form was significantly higher in the ET versus ST animals ($p<0.001$) whereas no significant differences were found for mature IL-1ß. WB of IL-1R tI showed one band at 65kDa which correspond to the reported molecular weight of IL-1R tI. No significant differences were found for IL-1R tI expression. In the bovine blastocyst, IL-1ß and IL-1R tI were predominately co-localized in the cytoplasm of trophectoderm cells. No differences in signal intensity were found between *in vivo* and control embryos.

Conclusion The expression of IL-1 beta in the apical cytoplasm adjacent to the glandular lumen and the higher expression of IL-1 beta proform found in the endometrium of ET animals points out to a higher protein translation which will be readily secreted into the uterine lumen in ET vs. ST animals.
Our findings suggest that the IL-1 system might exert an important role in the early embryo-maternal dialogue in bovine

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Age-dependent adaptive capacity of sires during prolonged heat stress

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Implications Prolonged heat stress has a negative impact on semen quality of bull sires. The older the bull the higher the percentage of discarded semen.

Introduction External factors may negatively influence the reproductive ability of sires especially after prolonged exposure. As in a case of overheating, overcooling, micro traumas and insulation etc. a temporary and sometimes persistent deterioration of the quality and quantity of semen often occurs. (Sokolovskaja I.I , 1973, Amerhanov H.A. 2011 Abilov A.I.2013).

Material and methods The work has been conducted on the grounds of The Head Centre of Animal Reproduction ("HCR"). 20 bulls of Holstein breed has been divided into 3 age-dependant groups. Bull sires at the age from 2 to 3 years (n=8), from 5 to 6 (n=4) and from 9 to 11 years (n=8) were studied during the heat stress caused by abnormally high average temperature of the air +33.14± 4.16 °C. In total 1324 ejaculates has been analyzed. Semen collected from the same animals before the extreme heat period started had been used as a control The volume of ejaculate (ml), sperm concentration per ml (billion/ml), motility before and after cryopreservation (%), total number of sperm in the ejaculate (billion), discarded ejaculates rate (%) measured. The study proceeded for 210 days conditionally divided into 4 stages by 49-56 days depending on cycles of spermatogenesis and temperature factor. The abnormally high air temperature in Moscow region lasted for 56 days sins 23-June-2010 to 18-Aug-2010.

Results It has been established that thermal stress has a negative effect on the functional state of blood-testis barrier. In the group of young bulls up to 3 years of age we saw an increase in the percentage of discarded ejaculates 32.8±2.4 vs 14.0± 2.0 in the control group ($P < 0.001$). For bulls of older age group (9-11 years) discarded semen rate was 36.3± 2.1 vs 18,0 ± 2,3% in the control ($P < 0.001$). In the group of bulls of 5-6 years of age the rejected semen rate was 27 +3.4 %. Bulls from this group has recovered spermatogenesis in one cycle. After cessation of heat stress in the group of bulls aged 2-3 years qualitative characteristics of the sperm recovered to the control level within 56 days. In the group of bulls aged 9-11 years even after the 2nd cycle of spermatogenesis (III stage) semen quality was not fully recovered. The rejected semen rate was 34.7 +2.2 %. The volume of ejaculates and concentration per ml has not been affected by temperature but depended from the age of the sire. After incubation in thermostat under +38°C for 5 hours the motility of semen decreased for 20-30% in every group of animals. Estimated amount of theoretical loss of cryoprotected semen from sires of the third group was 1916 doses and in first and second groups 879 and 787 doses, respectively. Under expected conditions of heat stress we suggest to collect bulls semen in the early morning. Air conditioning of breeding pens is also advisable.

Conclusion It was found that during heat stress period the rate of discarded ejaculates had a strong connection with the age of the animal. In the first group of bull sires (2-3 years of age) discarded rate raised up to 2 times vs. control. In the second group (5-6 years) the discarded rate raised to 9% vs. control The recovery period for first and second groups was 1 cycle of spermatogenesis. In the third group discarded rate raised up to 3 times.). The same group also required the longest recovery period (2 cycles of spermatogenesis). It was found that 36.6% of ejaculates was discarded in the third group of bulls (over 9 years of age) after recovery period We would assume that the heat stress has a deep negative impact on the spermatogenesis of older bulls.

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Reproductive performance on four Irish grass based dairy herds: associations with milk production and milk composition

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Implications The study shows longer calving to service and calving to conception intervals are both associated with higher milk production in early lactation. However, conception rate to 1st service was not associated with milk production. This would indicate that differential effects of milk production on reproductive function in dairy cows.

Introduction Reproductive efficiency is one of the key drivers of profit in grass-based dairy systems. In this context metabolic, endocrine and production changes that occur during the early *postpartum* period are key determinants of reproductive success. The relationship between reproductive performance of dairy cows and milk yield is complex and involves both genetic and metabolic mechanisms. The objective of this study was to examine the relationship between milk production variables and reproductive performance of grass-based dairy cows.

Material and methods Spring calved dairy cows (n=208) from 4 grass-based Irish herds were used in this study. Cows were milked sampled for milk composition once monthly. Monthly yields of milk, fat, protein and lactose were calculated for the first four months of lactation. Additionally, total yield from the adjusted 305 day lactation records was also calculated. The milk energy output of each cow was calculated for each of the first four months as the total production and on using the equation as defined by Tyrell and Reid (1965). Cows were inseminated according to individual farm practice and all cows were scanned for pregnancy at 30-50 days post AI and subsequently at 80 to 100 days post AI. This was subsequently confirmed by calving records in the following year. From these records a number of key reproductive variables including: calving to service interval, calving to conception interval, conception rate to 1st service and pregnancy rate for all services combined were calculated. The relationships between the binomially distributed dependent variable(s) conception rate to 1st service and overall pregnancy rate and the independent milk variables were analysed using logistic regression (SAS). The relationships between normally distributed independent and dependent variable(s) was analysed by regression analysis.

Results There was no association between total milk, protein, fat or lactose production for the 1st 4 months of lactation and any of the fertility variables measured (P>0.05). Significant associations were found between the yield of milk, fat and protein as well as milk energy output from individual months with conception rate to 1st service and overall pregnancy rate and these are presented in Table 1. There was an association between calving to service interval and milk yield in months 1, 3 and 4, fat yield in months 1, 2, 3 and 4 and protein yield in months 3 and 4 and total lactation milk yield (P<0.01). There was a positive association (P<0.05) between total milk yield and milk, fat and protein yields in months 1 and 4 with interval to calving to conception interval (odds ratio >1.0). There was a tendency (P <0.10) for a positive association of protein yield in month 1 with calving to conception interval. Longer calving to conception interval was associated with increased milk energy output in months 1, 2 and 4 (P<0.01) and in month 3 (P = 0.052).

Table 1 Association between milk traits and PD1 and PDFINAL

Fertility parameter	Variable	Odds ratio	95% CI	P value
Conception rate to 1st service	Milk kg month 3	1.096	1.017 - 1.180	P = 0.016
	Milk energy month 3	1.093	1.013 - 1.065	P = 0.003
	Protein kg month 3	14.861	1.756 - 125.801	P = 0.013
	Fat kg month 3	9.024	1.865 – 43.655	P = 0.006
	Lactose kg month 3	13.478	2.451 – 74.118	P = 0.028
Overall pregnancy rate	Fat kg month 3	0.039	0.002 - 0.616	P = 0.021
	Milk energy month 4	1.025	0.989 - 1.062	P = 0.067

Conclusion The findings of this study would suggest that the positive relationship between conception rate and production in the 3rd month of lactation when most cows are inseminated. However, both calving to service and conception interval were influenced by both milk yield and composition.

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