Genetic improvement in cattle – are we sacrificing reproduction in favor of production?

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Summary
Reproductive traits can range from lowly to moderately heritable. Genomic technologies provide a powerful tool for improving selection for traits that are lowly heritable, sex-linked, or not expressed until later in life. Therefore, as genomic technologies become a part of selection decisions, there is a critical need to understand how specific gene variants affect reproductive traits in cattle. Both classical quantitative genetics approaches and genomic approaches have identified genetic correlations between production traits and fertility. In dairy cows, single trait selection for milk production and associated inbreeding has resulted in a decline in conception rates over the last 60 years. Conversely, increases in body condition score at the time of calving in both beef and dairy cows are advantageously genetically correlated with decreased postpartum intervals to estrus. In beef cattle, the allele of μ-calpain (CAPN1) associated with increased meat tenderness is also associated with an increased postpartum interval to ovulation that could be detrimental to reproductive performance in the cow herd. However, in Charolais cattle, there was no genetic correlation between ovarian activity in females and proportion of adipose tissue in male carcasses, indicating that carcass traits can be improved without negatively impacting reproduction in the cow herd when selection is applied properly. Thus, there is a need for a systems based approach to understand how specific gene variants influence the overall physiology to ensure that selection pressure is applied uniformly for production, disease resistance, heat tolerance, and parasite resistance without negatively impacting reproductive efficiency in the cow herd.

Introduction
The economic value of reproduction to the commercial producer is five times greater than milk production or growth rate (Trenkle & Willham 1977, Randel & Welsh 2012), and selection
focused only on production traits can have negative consequences for fertility (Pryce et al. 2010, Cochran et al. 2013, Sugimoto et al. 2013, Wolcott et al. 2014). Perhaps the best example of this is the decline in fertility in dairy cows that has occurred as positive selection for milk production has been applied (Butler 1998, Lucy 2001). Milk production increased during the last 60 years as a result of this selection; however, conception rates declined precipitously during the same time. It is possible that assisted reproductive technologies simply mask these antagonisms and potentially allow the problems to increase. Therefore, care must be taken to ensure that selection for production traits does not result in the selection of gene variants that antagonize reproductive traits. With advancements in genomic technologies, it has become possible to understand the roles of specific genes on production and reproductive function and potentially counteract the antagonisms of these gene variants on fertility through marker-assisted selection.

Fortunately, female reproductive traits expressed early in life appear to be excellent candidates for predicting lifetime reproductive performance (Cushman et al. 2014, Johnston et al. 2014). For example, conceiving early in the first breeding season is associated with increased fecundity in beef cows (Lesmeister et al. 1973, Cushman et al. 2013a, Perry & Cushman 2013), and development of the reproductive axis is a genetically controlled process (MacLaughlin et al. 2001). Conversely, heifers with poor reproductive tract development prior to their first breeding season have decreased pregnancy rates, later calving dates, lower calf weaning weights, and decreased rebreeding performance (Holm et al. 2009). Johnston et al. (2009) reported that reproductive tract size in heifers was a heritable trait, and differences in endometrial gene expression between cows with differing levels of fertility have been identified (Minten et al. 2013). Thus, it is critical to understand how developmental genes that enhance growth and carcass traits are also influencing onset of reproductive cycles, development of the reproductive tract, and establishment of the ovarian reserve.

Genetic relationships amongst reproductive traits are generally favorable. Age at puberty and post-partum interval to first estrus are reproductive traits with moderate to high heritability that are favorably genetically correlated (Mialon et al. 2000, Morris et al. 2000, Cammack et al. 2009, Cushman et al. 2014, Johnston et al. 2014). This indicates that a set of genes may influence the onset of reproductive cycles both at puberty and in the post-partum period. These genes could contribute to early conception and increased fertility. For instance, selection for a decreased age at puberty in Angus heifers resulted in an increase in the pregnancy rate as they became mature cows (Morris et al. 2000), indicating that the onset of normal reproductive cycles and behavioral estrus are important components of fertility in domestic ruminants.

**Relationships between production traits and reproductive traits**

Genetic correlations between production traits and fertility have been reported for both beef and dairy cows. Bormann et al. (2006) reported an antagonistic genetic correlation between yearling gain and pregnancy rate in beef heifers where increasing growth rate of heifers decreased the fertility. Johnston et al. (2009) reported antagonistic genetic correlations of residual feed intake and meat color in steers with age at puberty in heifers. In dairy cows, days of productive life had an antagonistic genetic correlation with milk yield and fat yield, but calving interval, days to first service, and number of inseminations were favorably genetically correlated with days of productive life (Pritchard et al. 2013). Bastin et al. (2012) reported antagonistic genetic correlations between milk yield, fat yield, and protein yield and days open. Adipose is an important tissue that provides endocrine regulation of reproductive function. The genetic correlations for specific fatty acids with days open changed with days in milk. This was likely explained by the physiological status of the cows, where cows in negative energy balance during
early lactation may mobilize different fatty acids from fat depots compared to fatty acids available during positive energy balance status later in lactation. Genes that regulate deposition and mobilization of fat depots will most likely provide key genetic markers for applying selection pressure that is balanced between production and reproduction (Galic et al. 2010).

A putative functional polymorphism near the pleiomorphic adenoma gene 1 (PLAG1) was associated with decreased serum insulin-like growth factor-1 and fat depth (Fortes et al. 2013). This single nucleotide polymorphism (SNP) was associated with increased net food intake, weight and hip height, but resulted in a delay in age at puberty. Other polymorphisms in seven genes associated with production traits were used to examine the variation in reproductive traits (Collis et al. 2012); results showed a number of alleles associated with favorable production traits were not favorable for reproductive traits. Among these, the allele of CAPN1 that was associated with increased meat tenderness was associated with longer post-partum anestrous intervals. Not all of these relationships are antagonistic. While cow longevity was negatively genetically correlated with carcass conformation, it was advantageously genetically correlated with birth weight, weight at 120 days of age, weight at 210 days of age, cold carcass weight, fatness, and meat color (Varona et al. 2012). Furthermore, the genetic correlation between calving day and weight adjusted to 550 days of age was near zero, and selection for increased post-weaning gain did not negatively impact development of the reproductive tract and ovaries in Nelore heifers (Forni & Albuquerque 2005, Monteiro et al. 2013).

Applying genomic technologies to reproductive traits

Many reproductive traits are lowly to moderately heritable, sex-limited, binomial in nature, or expressed late in life; making traditional selection methods difficult (Cushman et al. 2008, Cammack et al. 2009). Genomic technologies have the ability to identify chromosomal regions associated with reproductive traits in cattle (Fortes et al. 2010, Pryce et al. 2010, Snelling et al. 2012, Sugimoto et al. 2013). However, these studies have identified very few SNPs that reach significance when corrected for multiple testing, and may indicate that these traits are pleiotropic in nature (Fortes et al. 2010, Fortes et al. 2013). To circumvent this difficulty in identifying specific genes, investigators have used the results of genome-wide association studies to estimate effects using all the available genotypes (Allan & Smith 2008). Pryce et al. (2010) reported few SNP from the BovineSNP50 BeadChip associated with fertility traits in dairy cows when corrected for multiple testing; however, they were able to identify unfavorable genomic correlations between milk production and fertility traits. This demonstrates the need to identify the underlying gene variants and their effects on whole animal physiology, because applying genomic selection without understanding the underlying genes can cause antagonistic interactions in exactly the same way that single trait selection can (Allan & Smith 2008, Snelling et al. 2013).

Snelling et al. (2012) reported no significant SNPs for age at puberty or heifer pregnancy rate when corrected for multiple testing (Figs. 1 and 2). Only one SNP located in the muscle gene Titin (TTN) was significant for antral follicle count when corrected for multiple testing (Fig. 3). This SNP is in the non-coding region of Titin and the functional polymorphism influencing follicle count could be in Titin or another gene that is in linkage disequilibrium with this SNP. Other polymorphisms in Titin have been associated with marbling in Japanese Black cattle (Yamada et al. 2009, Watanabe et al. 2011) but the degree and direction of the relationship between these markers and the one associated with antral follicle count are unknown. Further research is needed to ensure that using genetic markers in Titin for selection can do so without negatively impacting the ovarian reserve in the cow herd.
Fig. 1. Manhattan plot of genome-wide SNP association with age at puberty using the Illumina BovineSNP50 beadchip. No markers reached significance when adjusted for multiple testing (Dashed red line).

Fig. 2. Manhattan plot of genome-wide SNP association with heifer pregnancy rate using the Illumina BovineSNP50 beadchip. No markers reached significance when adjusted for multiple testing (Dashed red line).

Fig. 3. Manhattan plot of genome-wide SNP association with antral follicle count using the Illumina BovineSNP50 beadchip. A single marker in the non-coding region of Titin exceeded significance when adjusted for multiple testing (Dashed red line).
The main reason that few of these studies have identified major gene effects on fertility is the need for animals to be able to reproduce in order to pass their genes to the next generation. The majority of the segregating gene variants that affect fertility will likely result in sub-fertility, because those that result in infertility will be rapidly lost from a population if the carriers fail to reproduce. However, gene variants that result in reproductive failure only when in a homozygous state can remain in the population if they convey a selection advantage to heterozygous animals. Inbreeding, which reflects homozygosity, has a detrimental effect on fertility while effects of heterosis, arising from crossbreeding are favorable. Declining fertility of dairy cows may be at least partially due to increased levels of inbreeding, coinciding with intense selection for increased milk production using a smaller number of sires through artificial insemination. In a long-term study of lifetime production, Cundiff et al. (1992) demonstrated heterosis for pregnancy, calving, and weaning rates that resulted in crossbred cows averaging at least one more calf weaned over their lifetime than contemporary straightbred cows. Using the BovineSNP50 BeadChip, Snelling et al. (2012) demonstrated detrimental effects of genomic inbreeding on heifer pregnancy rates, indicating that heifer that were homozygous for many low frequency alleles were less likely to become pregnant.

Specific genes and structural variants resulting in an advantage for heterozygotes have been reported. Kadri et al. (2014) used the BovineSNP50 BeadChip to identify a 660-Kb deletion on bovine chromosome 12 in Nordic Red Cattle that was associated with increased milk yield in heterozygous animals but resulted in embryonic lethality in homozygous animals. Other genes having heterozygous advantage include growth differentiation factor 9 and bone morphogenic protein 15 where polymorphisms cause reproductive failure due to improper follicular development in homozygous ewes but result in increased prolificacy in heterozygous ewes (Galloway et al. 2000, Hanrahan et al. 2004). Identification of genomic regions with heterozygous advantage and homozygous infertility allow development of mating schemes which leverage the heterozygote productivity, while limiting the infertility of homozygotes. Furthermore, genotyping young animals identifies where individual animals will fit into such a mating strategy.

Systems based investigations of the genetic changes

Researchers have addressed the issues of identifying polymorphisms that result in subtle changes in fertility by dissecting specific component traits and identifying candidate genes that could be influencing these traits, based on a priori knowledge of the biological system. Members of the transforming growth factor-β (TGFβ) super-family are involved in growth and development, with several members (e.g. myostatin and bone morphogenic protein 8B) and their receptors being identified as genetic markers for carcass or growth traits (Sellick et al. 2007, Esmailizadeh et al. 2008, Cao et al. 2013). While other members of the TGFβ family (e.g. anti-Müllerian hormone, growth differentiation factor 9, and bone morphogenic protein 15) are involved in fecundity and fertility (Cushman et al. 2002, Gigli et al. 2005, McNatty et al. 2005, Tang et al. 2013). Similar results are observed for members of the WNT family that have been reported to be associated with carcass traits in poultry (Lu et al. 2012), and are also crucial for proper development of the reproductive tract and the ovaries (Vainio et al. 1999). These genes are expressed in numerous tissues. Thus, there is a need for a systems based approach to understand how specific polymorphisms influence whole animal physiology.

Several studies have identified genes in the gonadotropin signaling pathway and steroidogenic synthesis pathway as excellent candidates to influence the initiation of reproductive cycles (Fortes et al. 2010, Sugimoto et al. 2010, Cushman et al. 2013b, Homer et al. 2013, Sugimoto
Polymorphisms in the gonadotropin-releasing hormone receptor (GNRHR) were associated with age at first calving in beef heifers and with expression of behavioral estrus in dairy cows (Cushman et al. 2013b, Homer et al. 2013). Expression of behavioral estrus is a trait that is associated with increased fertility in cattle (Perry & Perry 2008). This increase in fertility is due to increased preovulatory estradiol concentrations in the cows that express behavioral estrus (Perry et al. 2005, Jinks et al. 2013). In both beef cows and dairy cows, estrogen synthesis pathways and estrus behavior have been associated with genetic markers for reproductive traits (Fortes et al. 2010, Cochran et al. 2013, Homer et al. 2013). Cochran et al. (2013) reported 40 SNP that were associated with daughter pregnancy rate in dairy cows. Of these, 11 were negatively associated with yield traits, leaving 29 that were not associated with yield traits. This demonstrates the availability of genetic markers with potential to improve fertility by selection without compromising production.

Body composition also influences the onset of reproductive cycles in replacement heifers and post-partum cows. A number of studies have reported a favorable genetic correlation between body condition score and the onset of reproductive cycles in heifers and cows (Mialon et al. 2001, Zink et al. 2011, Johnston et al. 2014). Selection for low residual feed intake in replacement beef heifers is associated with later calving, most likely due to an increase in lean mass (Randel & Welsh 2012). These authors concluded that it might not be possible to improve feed efficiency without sacrificing reproductive efficiency; however, this is exactly the situation where genomic approaches can improve selection decisions. A set of genetic markers within and near the adipokine, chemerin (RARRES2), on bovine chromosome 4 were associated with residual feed intake, average daily gain, and average daily feed intake as well as average fat thickness and marbling (Lindholm-Perry et al. 2012). There were cases where the allele that was associated with increased feed efficiency was associated with increased adjusted fat thickness, breaking the established relationship of improved feed efficiency with increased leanness. Thus, indicating that by using specific genetic markers it may be possible to improve feed efficiency without compromising body composition, thereby maintaining the ability to attain puberty at an acceptable age in heifers. Along these lines, Mialon et al. (2001) reported no genetic correlation between female ovarian activity and the proportion of adipose tissue in male carcasses in a population of Charolais cattle.

Selection for growth and carcass traits can have other consequences as well. Heifers that experience dystocia have an increased risk of calf mortality, retained placenta, prolonged re-breeding interval, and death (Laster et al. 1973, Bennett & Gregory 2001a). Positive correlations of dystocia with calf birth weights, 200-d weights, and post-weaning gain suggest that selection for carcass yield can have negative implications on reproductive performance in the cow herd due to increases in dystocia (Bennett & Gregory 2001a, Bennett & Gregory 2001b). A genome-wide association study identified chromosomal regions associated with calving traits in Holstein-Friesian cows (Purfield et al. 2013). A polymorphism in the hedgehog interacting protein (HHIP) gene that is associated with stature in humans was associated with direct calving difficulty, although polymorphisms in the bovine HHIP gene were not associated with stature in cattle (Pryce et al. 2011). Hedgehog signaling is interesting because improper signaling can lead to anovulation in mice (Ren et al. 2009) and can alter development of the female reproductive tract (Migone et al. 2012, Ren et al. 2012). This again shows the complexity of the biology where small perturbations in a signaling pathway could have pleiotropic effects in a multitude of tissues.

Because of these pleiotropic effects in multiple tissues, it is necessary to move beyond association studies and use systems based research to understand how these polymorphisms function. An excellent example of this is the growth hormone (GH1) polymorphism that is the
proposed causative mutation for the miniature condition in Brahman cattle. These cattle have serum growth hormone concentrations that are greater than normal, but insulin-like growth factor-1 concentrations that are lower than normal (Hammond et al. 1991). This led investigators to first propose that this was due to a polymorphism in the growth hormone receptor (GHR); however, treatment with exogenous growth hormone caused an increase in serum insulin-like growth factor-1 concentrations (Chase et al. 2011). Subsequent investigation demonstrated the presence of a polymorphism in GH1 that resulted in an alternate form of growth hormone with 60% of the activity of native GH1 in a luciferase reporter system in a transfected Chinese hamster ovary-derived cell line (McCormack et al. 2009). The polymorphism caused a decrease in antral follicle numbers, but did not negatively impact fertility in cattle (Chase et al. 1998). This example demonstrates how sound physiological studies can aid in identifying and understanding functional polymorphisms. However, this is an extreme phenotype that is easy to identify by the change in body stature.

For every success in identifying functional polymorphisms, there are results that lead to more questionable conclusions. For example, a study in dairy cattle identified a polymorphism in the ionotropic glutamate receptor AMPA1 (GRIA1) that resulted in a decrease in the number of follicles detectable by ultrasonography, a change in the timing of the luteinizing hormone surge, and a decrease in conception to artificial insemination. When the alternate form of the receptor was transfected into immortalized murine hypothalamic GT1-8 cells, the release of gonadotropin-releasing hormone in response to treatment with glutamate in vitro was attenuated (Sugimoto et al. 2010). However, when the GRIA1 polymorphism was tested in a population of repeat-breeder beef cows, it was not associated with the number of antral follicles present at necropsy (Cushman et al. 2013b). These conflicting results in association studies do not prove or disprove the functionality of the polymorphism. There are several explanations for the differences between the cattle populations. The beef cow population could have another polymorphism in GRIA1 or an associated gene that counteracts the effects of the primary GRIA1 polymorphism. Alternatively, the effect of the GRIA1 polymorphism could be more dramatic in lactating dairy cows where glutamate availability might be more limited due to its conversion to pyruvate in a negative energy balance condition. It will take complex studies of transcriptomics, proteomics, and metabolomics in numerous tissues to understand the functionality of these polymorphisms in the whole animal.

**Conclusions**

Are we sacrificing reproduction for production? Possibly, in the vast majority of cases production traits show antagonistic correlations with reproduction; however, there are situations where the relationship is neutral. There are hints of genetic markers that could be positive for both production and reproduction. These genetic markers with neutral and positive relationships can be used to our advantage to develop more balanced selection programs. In cattle, the dairy industry has adopted the use of genome-wide associations to select for daughter pregnancy rate, because historical selection for milk production has clearly reduced fertility. *Bos indicus* cattle provide heat tolerance and parasite resistance that make them particularly suited for regions of the world where *Bos taurus* cattle do not perform as well. However, the delayed puberty and decreased conception rates in *Bos indicus* cattle have led to research to identify genetic markers to aid in improving reproductive rates in these breeds. At this point, the need to select for fertility in *Bos taurus* breeds of beef cattle is not a perceived issue for most producers because conception rates are acceptable. However, continued genetic and genomic
selection solely for production traits, without considering accumulated inbreeding or correlated responses in fertility, could eventually reduce fertility in these breeds as has happened in dairy breeds. Therefore, the identification of genetic markers for fertility and an understanding of how gene variants used to improve production traits also influence fertility are important for the continued improvement of cattle.

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