TAKE HOME MESSAGE

- Genomic selection was a critical tool for stopping the decline in Holstein cow fertility, and the fertility of the Holstein cow is now slowly but steadily improving.
- You can improve fertility in your herd by using bulls with high genomic breeding values for fertility traits, and you will see those changes in one generation.
- The availability of low-cost, high-density genetic marker panels and whole-genome sequence data has allowed us to identify genes associated with large effects on fertility, and to dissect the biology of gametogenesis, fertilization, and development.
- Continued development of reproductive technologies such as sexed semen, oocyte pickup, and embryo transfer has provided dairy farmers with new tools to increase rates of genetic gain.
• Other current and anticipated advances in reproductive management, including timed artificial insemination and development of remote sensing technologies, will allow farmers to improve the fertility of their herds and increase their profitability.

WHAT WAS THE STATE OF DAIRY COW FERTILITY BEFORE GENOMICS?

Before the introduction of genomic selection in 2009 (Wiggans et al., 2017), the U.S. Holstein breed had experienced a prolonged decline in fertility due to the negative association with yield (Hansen, 2000). Daughter pregnancy rate had decreased from a high of 39.5% in 1959 to a low of 24.1% in 2005 (Council on Dairy Cattle Breeding, 2020). This represents an increase of 1.3 days open per year. The situation was not acceptable to farmers, who tried many different strategies, such as advanced reproductive technologies, crossbreeding, and switching breeds entirely. Fertility was first added to the Lifetime Net Merit selection index in 2003 with a weight of 7% (VanRaden and Seykora, 2003). Today, the fertility traits (daughter pregnancy rate, heifer conception rate, and cow conception rate) receive 10% of the weight in the index (VanRaden et al., 2018).

A major challenge when trying to improve fertility through genetics is the question of how to measure it. For genetic improvement, we want to record observations for as many animals as we can, as cheaply as possible. The DPR evaluations are based on observations of days open, while heifer (HCR) and cow conception rates (CCR) are based on breeding and pregnancy-check data. The low heritabilities of these traits indicate that the environment plays a much bigger role in their expression than does genetics. Among other things, each of these traits is affected by management decisions made by the farmer. Age at first calving is also evaluated as early first calving (EFC), (Hutchison et al., 2017) but may be a more general measure of the onset of puberty than fertility per se. This leaves us with an important question: are our measures of fertility very good? Are we measuring things that have a strong correlation with reproductive physiology, or are we simply measuring things that are easy to record?
HOW DID GENOMICS CHANGE THINGS?

The genetic trend for three fertility traits in U.S. Holsteins – daughter pregnancy rate and heifer and cow conception rates – are shown in Figure 1. The first animals to be genotyped were bulls. As the cost of testing decreased, many farmers began routinely testing their cows and there are now more than 3.7 million genotyped Holsteins in the National Cooperator Database maintained by the Council on Dairy Cattle Breeding (Bowie, MD). With the help of genomic selection, we are improving genetic merit for fertility faster than we were before that tool was available.

However, the rapid adoption of genomic selection has resulted in some big changes in genetic improvement programs. Perhaps the most notable of these is that the generation interval has decreased by almost half (García-Ruiz et al., 2016), which means that the rate of genetic gain has increased substantially. This is driven largely by a shift from predominant usage of 5-7-year-old progeny-tested bulls in AI programs to usage of young bulls under 4 years of age (Hutchison et al., 2014). One consequence of these changes is that few bulls receive a conventional progeny test anymore. By the time second-crop daughters come in, a bull is 2-3 generations behind the young bulls in the population.

Some people are worried that the genetic evaluations are not as stable now as they used to be, meaning that bull evaluations can change a lot from one genetic evaluation run to another, but that is not the case. Early in the development of genomic selection, there were sometimes large changes from one run to another, but the statistical methodology is now mature and such changes are rarely seen. As a reminder, there were sometimes large changes in traditional (non-genomic) evaluations when, for example, lots of daughter records were added at one time to a bull’s proof.
You might wonder if going faster is actually better – we went in the wrong direction for a long time, how can we be sure that we are actually on the right path today? A recent study of 4,445 Holsteins from 4 commercial dairies showed that animals with genomic PTA for DPR in the top quartile had higher pregnancy rates, lower days open, and fewer services to conception than cows with PTA in the bottom quartile (Lima et al., 2020). Similarly, cows with PTA for DPR > 1 (n = 1,285) had higher pregnancy rate at first service, fewer services per conception, and fewer days open that cows with a DPR ≤ -1 (n = 989) (Ortega et al., 2017a). These results are important because they confirm that animals predicted to have high fertility generally do, and animals predicted to perform poorly usually do.

WHERE ARE WE TODAY?

Knowledge of the Molecular Genetics of Reproductive Biology is Advancing Rapidly

Improved breeding values may be the most visible product of the genomic revolution in dairy cattle but there is much more to the story than that. Over the last 12 years, we have dramatically improved our understanding of the reproductive biology of the cow. With this knowledge has come identification of the specific genes controlling reproductive processes. Candidate markers were identified and validated in genes associated with reproductive processes (Cochran et al., 2013a; b), which resulted in the addition of 39 SNP to the genomic evaluation panel used by the Council on Dairy Cattle Breeding (CDCB; Bowie, MD). Addition of these markers increased the accuracy of prediction by 0.20% (Ortega et al., 2016), which may seem small, but the increase from 50 k to 777 k SNP genotypes increased accuracy by only 0.40% (VanRaden et al., 2013). This shows that a carefully targeted approach to identify new DNA markers can be much more efficient than selection of markers without using functional information. A single change in COQ9 had favorable effects on cellular oxygen metabolism, body weight changes, and ovarian function (Ortega et al., 2017). We also now know that the use of bulls only, cows only, or a mix of the two in association studies identify different sets of genes that often do not overlap (Gaddis et al.,
2016). The use of embryo transfer continues to grow, and there is evidence that several aspects of that process are under some degree of genetic control (Jaton et al., 2016; Parker Gaddis et al., 2017), although genetic effects are very small for some traits.

As the example of COQ9 illustrates, it is likely that much of the genetic variation affecting reproduction is not caused by mutations affecting function or expression of genes that evolved specifically for a function in the embryo or reproductive tissues, but rather that play a role in both reproductive and non-reproductive cells alike. Embryo-lethal mutations in housekeeping genes, such as the mutation in CWC15 responsible for the JH1 fertility haplotype in Jerseys (Sonstegard et al., 2013), are a reminder that lethal recessive alleles are an important cause of infertility and that inbreeding reduces reproductive function (Martikainen et al., 2018).

There is a Renewed Appreciation of Andrology

After many years of dormancy, research into the reproductive biology of the male is accelerating. At one level, the race for obtaining semen from the best bulls is increasing the pressure for early puberty in bulls. In addition, however, there are opportunities for improving bull fertility through genetic selection, management or post-harvest processing of semen. The use of sexed semen has become commonplace in commercial dairy production (e.g., Burnell, 2019), but recent research suggests that sorted semen have different motility profiles and reduced rates of embryonic development than conventional semen (Steele et al., 2020). This is consistent with findings of Ortega et al. (2018) that bulls with high (favorable) sire conception rates (SCR) produce more fertilized oocytes and fewer degenerate embryos than do low-SCR bulls. Interactions between the sperm and the female reproductive tract are crucial for maintaining sperm survival in the reproductive tract (Pollard et al., 1991; Kumaresan et al., 2019). The availability of genotypes for virtually all bulls entering artificial insemination service is also being used to identify causal variants for genetic differences in fertility among bulls (Hiltpold et al., 2020). Lastly, recent
advances in flow cytometry and machine learning may increase the accuracy of evaluating bull fertility
(Bucher et al., 2019).

Dairy Farmers Have Rapidly Embraced New Reproductive Tools

Sexed Semen. The rapid adoption of sexed semen as a common tool in reproductive management has
been driven by rapid improvements in the technology. While initially used principally on heifers due to
lower fertility of sexed semen (e.g., Norman et al., 2010), it is not commonly used on heifers and older
cows, and there is little difference in fertility between conventional and sexed semen. This has allowed
dairies to adopt more complex breeding strategies because they are assured of a surplus of heifer calves as
replacements.

Beef-on-Dairy. The increasing use of beef bulls on dairy cows to produce higher-valued calves for the
packing sector is not, strictly speaking, a fertility issue, but it is enabled by the surplus of heifer calves
resulting from widespread use of sexed semen. Sales of beef semen have increased rapidly in the US over
the last few years (Geiger, 2020; National Association of Animal Breeders, 2020), which can increase
farm profitability (Ettema et al., 2017) and is consistent with anecdotal reports of a shift in breeding
strategy.

Who Benefits? There is some evidence that farmers with larger herds are more likely to adopt new
technologies than those with smaller herds (Gargiulo et al., 2018), which appears to be driven largely by
labor costs on large farms. This leads one to wonder if new reproductive technologies are just more tool
that benefits the farmer with 1,200 or 12,000 cows, and not the producer with 120 cows. However, one
notable difference between precision technologies, such as automated milking systems, and beef or sexed
semen is that the former requires substantial capitalization and the latter do not. It is certainly possible
that a very large dairy may negotiate a slightly more favorable per-unit price due to volume purchases, but it is not clear that such advantages make this semen off-limits for the smaller farm.

WHERE ARE WE GOING?

What Should We Do About Genetic Diseases? It is inevitable that undesirable genetic defects will arise in any population, and the widespread use of artificial insemination in dairy cattle means that we will sometimes have to manage such conditions (Cole et al., 2016). Currently, there are 20 such haplotypes being monitored in the US dairy population (Cole et al., 2018), and there are more being reported in other countries (e.g., Fritz et al., 2018; Häfliger et al., 2020). Due to the complexity of interpreting these data, genetic diseases are best managed using a computerized mating system (Cole, 2015; Upperman et al., 2019). However, this is always a moving target because, as one disease or another is brought under control, new ones will arise. MacArthur et al. (2012) have reported that human genomes typically contain ~100 genuine loss-of-function variants, with ~20 genes being completely inactivated. Statistics are likely similar in the bovine, and a lethal mutation can rapidly increase in frequency when it is inherited by a genetically elite bull. For example, the Holstein bull Carlin-M Ivanhoe Bell (007HO00543; HOUSA000001667366), who has more than 80,000 offspring, was the carrier of both the bovine leukocyte adhesion deficiency (Shuster et al., 1992) and complex vertebral malformation (Agerholm et al., 2001) defects.

How Should We Introduce or Multiply Desirable Genes? There is growing interest in strategies for spreading desirable genetic variants, such as the slick mutation in the prolactin receptor gene (Hansen, 2020a) and polled allele (Mueller et al., 2019), through dairy cattle populations. As is the case with beef-on-dairy, this is not a exactly an advance in reproductive biology, but most strategies for introgressing these alleles incorporate IVF and embryo transfer (ET) at some stage in the process (Cole, 2019). In that sense, improvements in embryo technologies which increase the use of ET as a genetic selection tool will
also benefit these schemes (Hansen, 2020b), and have the potential to increase the value of carrier embryos in the ET marketplace. Gene editing is now routine and the advent of livestock with mutations designed by humans is on the near horizon. How such animals are accepted by the public will be an important determinant of how quickly the technology sees application in the United States.

**New Reproductive Traits.** The advantage of measuring traits like CCR or DPR is that millions of records can be obtained relatively easily using records collected on farms. Unfortunately, these traits are affected to an extraordinary degree by environment, including management decisions of the dairy farmer, and the heritabilities of these traits is very low. Other reproductive traits may be more heritable. As pointed out as far back as 8 years ago (Boichard and Brochard, 2012), development of new devices for measuring reproductive physiology of cows may make it feasible to incorporate new traits into selection indices for reproduction. It remains to be seen whether other physiological measurements of reproduction can be found that are both more heritable than traits like CCR or DPR and feasible for wide-scale measurement. Concentrations in blood of anti-Müllerian hormone are very heritable (Nawaz et al., 2018) and the intervals from calving to estrus and calving to elevated progesterone concentrations were moderately heritable (Ismael et al., 2015; Tarekegn et al., 2019). In contrast, heritabilities were low for duration and strength of estrus (Ismael et al., 2015), progesterone concentrations during the luteal phase (Tarekegn et al., 2019) and embryo survival after ET (Parker-Gaddis et al., 2017).

**More Embryo Transfer?** While ET has been common in some parts of the world, notably in Brazil where the large yield of oocytes from *Bos indicus* increases the efficiency of in vitro production systems, ET is now increasing in importance on US dairies. While mostly limited to high-end breeders in the past, the potential of ET to increase the number of offspring produced by genetically elite females is of growing importance (e.g., Fleming et al., 2018). What will be essential to make use of ET more widespread is to reduce the costs of embryo production and increase the effectiveness of the procedure for achieving
pregnancies (Hansen, 2020b). A recent reported advance in cryopreservation of embryos produced in vitro (Gómez et al., 2020), if repeated, could be an important step in that direction.

**Should We Continue to Depend on Reproductive Hormones in the Long Run?** Timed AI, which is now very common on US dairy farms, is dependent on the use of exogeneous reproductive hormones. There is some evidence that effectiveness of hormonal interventions to increase fertility depends on interactions with specific mutations in genes associated with reproduction (Zolini et al., 2019). It is possible, therefore, that timed AI, like any other management intervention, could lead to a change in frequency of specific alleles.

While American consumers have generally been more accepting of biotechnological innovations in the food chain, a change in consumer sentiment could rapidly change the regulatory landscape (Ufer et al., 2019). Timed AI offers several advantages to the farmer including reducing the need for estrus detection and improvement in fertility as compared to induced estrus (Carvalho et al., 2018). It can also be considered a method for improving animal welfare since, as pointed out by JEP Santos (personal communication), an open cow often ends up as a dead cow. It is likely that application of precision agricultural technologies to dairy cattle management will lead to new methods for detecting estrus and pregnancy diagnosis. If this occurs, some of the rationale for timed AI will dissipate.

**CONCLUSIONS**

Dairy cow fertility has been steadily improving since the introduction of genomic selection in 2008. In addition to more accurate genetic evaluations, genomics has led to a deeper understanding of gamete and cow physiology, as well as enabled more sophisticated strategies for herd management. While we are unlikely to see another tool that provides such a rapid change in cow performance, we will continue to benefit from discoveries enabled by genomic selection for a very long time.
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Figure 1. Trends in U.S. Holstein breeding values (BV) for daughter pregnancy rate (DPR; red lines), heifer conception rate (HCR; blue lines), and cow conception rate (CCR; black lines) for bulls (solid lines) and cows (broken lines) for animals born 1957—2019.¹

¹Data were taken from the Council on Dairy Cattle Breeding’s August 2020 genetic and phenotypic trends page (https://queries.uscdcb.com/eval/summary/trend.cfm).